Exploring how land use may influence the export, composition, and reprocessing of dissolved organic carbon in peat-rich catchment drainage

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Abstract

Organic soils play an important role in the fluvial carbon cycle through production and export of dissolved organic carbon (DOC) from the breakdown of plant material. However little is known about the changes in organic soil-derived fluvial DOC composition and its potential impacts on in-stream DOC breakdown dynamics. This research explores how aquatic DOC composition is influenced by land use, and how the biological utilisation of DOC may be influenced by its composition. Specifically, this research focusses on peat-rich catchments, subject to disturbance for wind farm construction to:

1. explore for how long the disturbance from wind farm development affects water chemistry in peaty catchments through analysing the long-term trends in fluvial DOC and nutrient concentrations in the draining streams;

2. assess if different wind farm-associated land uses, (construction and deforestation), exert different influences on fluvial DOC quantity and composition in peatland catchments;

3. investigate relationships between total dissolved iron (Fe) concentration [Fe] and concentrations of DOC and nutrients (phosphorus and nitrate), as this interaction may ultimately shape the DOC biodegradability during fluvial transport.

4. quantify how much DOC is biodegraded in peatland streams and what controls this.

To examine the wind farm disturbance on fluvial DOC concentration, [DOC], and spectrophotometric composition, streamwater samples from five peatland catchments draining the south of the Whitelee wind farm in Scotland were analysed monthly from 2014 to 2016. To further understand the impacts of wind farm-associated land uses, spatial and temporal variation of DOC concentration and composition were assessed between sub-catchments D-WF and D-FF within a small catchment, Drumtee. D-WF was draining the wind farm construction areas and D-FF draining the felled forestry. The concentrations of soluble reactive phosphorus, [SRP], and total oxidised nitrogen, [TON], were also measured.

Using existing and data new to this study, a long-term [DOC] increase (2006 - 2016) was observed in the Whitelee catchments since the start of wind farm development (October 2006, with the associated felling activities starting in November 2006). The increase may be a result of wind farm long-term disturbance, by generating more DOC which could be exported when discharge increases. Immediate impacts from wind farm were observed, with [DOC] and [SRP] increasing quickly after the construction began at the catchments most affected by the original wind farm development and its extension. Within Drumtee catchment (WL13), greater mean [DOC] was observed in D-FF than D-WF. This may suggest

that wind farm-related deforestation led to more peat decomposition and greater DOC release than the construction activities.

DOC is dominated by humic substances (HS), which generally can be further divided into humic acids (HAs) and fulvic acids (FAs). In WL13, DOC was less aromatic and humic than the other Whitelee catchments, with smaller $SUVA_{410}$ (an indicator for more-complex aromatic C) and larger E_2/E_4 (an indicator for proportion of humic substances) ($p < 0.05$ for both parameters). This could be a result of shorter water retention time and smaller [TON] in WL13 leading to less DOC biodegradation. Moreover, within-catchment difference of DOC composition was observed in WL13, with smaller $SUVA_{410}$ and larger E_2/E_4 indicating less DOC humification in the felled catchment ($p < 0.05$ for both parameters). This is interpreted related to different land use effects, as 'younger' DOC was produced from the decomposition of residual forest branch and leaves in the felled area, or 'older' DOC was released from the deeper peat in the wind farm areas. In addition, DOC in the two sub-catchments showed inverse changes during the dry periods, and one possible reason could be the different DOC sources from soil with low flow due to land use differences.

Total dissolved [Fe] measured in the fluvial samples allowed analysis of the interaction between dissolved Fe and DOC, and nutrients in fluvial systems. A positive significant relationship was found between dissolved [Fe] and [DOC], supporting interpretation that humic substances (HS) maintain Fe^{3+} in soluble state during transport. Fe-DOC complexation may be particularly facilitated by fulvic acids (FAs) than humic acids (HAs), supported by the relationships between Fe/DOC (Fe and C-DOC molar ratio) and E_2/E_4, and E_4/E_6 (FAs:HAs).

However, high [Fe] and occurrence of Fe oxides may limit DOC export by co-precipitation. This interaction is important as it may limit DOC export, impact terrestrial DOC characteristics and thus aquatic bioavailability. In addition, Fe-DOC complexation may increase P export and the Fe^{2+}-Fe^{3+} oxidation mediate TON reduction.

The in-stream breakdown dynamics of compositionally-different DOC were considered through incubations with water samples from Drumtee tributaries in autumn and early winter of 2015, and late winter and summer of 2016. Although [DOC] was higher in Drumtee than many other aquatic systems, only 2.9 – 12.1 % of the total DOC was biodegradable during 21 days. However, when normalized by the incubation length breakdown rates were comparable to other studies. A general seasonal variation of biodegradable DOC (BDOC) was observed across the sites, with more lost in late winter

and summer and less in autumn and early winter. Spatially, in autumn and early winter similar BDOC % loss was observed across sites, while in late winter and summer larger BDOC % removal was found in D-WF1 and WL13.

Weekly measurement of DOC composition and nutrient concentrations during the incubation revealed the dynamic process of DOC biodegradation. Protein-like fluorescence component C6 and TON changed actively with BDOC removal, suggesting C6 may be an important BDOC pool, and TON may provide substrate for the labile DOM to form. Initial DOC composition (SUVAs and C6) and nutrient availability seem important controls on BDOC loss. SUVAs showed negative correlations with BDOC which may because SUVAs represented the humic and refractory DOC pools. C6 and nutrients were all positively related to BDOC loss, and is likely due to their support for DOC consumption in different ways. The relationships help to understand the observed seasonal and spatial variation of Drumtee BDOC loss. The inter-stream difference of DOC composition may reflect the influence of wind farm-associated land use differences on in-stream DOC reprocessing and fate.

The understanding generated by this research advances knowledge of connecting the terrestrial and fluvial C cycle subject to land use changes. It reveals that wind farm development may have caused [DOC] increase. Particularly, deforestation can lead to larger fluvial [DOC] but with less refractory fractions than construction activities. DOC concentrations and composition can vary over space and time and in response to land use, and in turn this 'inherited' composition can influence the fate of DOC in aquatic systems.

Table of Contents

List of Tables

List of Figures

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Author's Declaration

I declare that the work outlined and described in this thesis has been carried out by myself unless otherwise acknowledged. This thesis is completely my own composition and has not, in whole or part, been submitted for any other degree at this or any other university.

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Abbreviations

Abs.	Absorbance
ANOVA	One way analysis of variance
BDOC	Biodegradable DOC
C	Carbon
CDOM	Chromophoric dissolved organic matter
CH_4	Methane
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
DI	Deionized
DIC	Dissolved inorganic carbon
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
D-FF	Drumtee-Felled forest
D-WF	Drumtee-Wind farm
C1 – 6	PARAFAC components 1 - 6
EEM	Excitation-emission matrix
E_2/E_4	The ratio between UV-visible absorbance at 254 nm and 410 nm
E_4/E_6	The ratio between UV-visible absorbance at 410 nm and 665 nm
FA	Fulvic acid
FDOM	Fluorescent DOM
Fe	Iron
$FePO_4$	Ferric phosphate
Fe/DOC	Dissolved Fe and DOC molar ratio
Fe/P	Dissolved Fe and SRP molar ratio
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
g m^{-2} yr^{-1}	Grams per metre squared per year
g m^2 $month^{-1}$	Grams per metre squared per month
Gt	Gigatonne = 10^9 t
HA	Humic acid
HCO_3^-	Bicarbonate ion
HMW	High-molecular-weight
HS	Humic substances

HY	Hydrological year
LMW	Low-molecular-weight
MPE	Maximum probable error
mg l^{-1}	Milligrams per litre
Mn^{2+}	Manganese ion
m s^{-1}	Metres per second
Mt	Megatonne = 10^6 t
m^3 yr^{-1}	Cubic metres per year
N	Nitrogen
NO_2-	nitrite
NO_3-	nitrate
OC	Organic carbon
OM	Organic matter
P	Phosphorous
PARAFAC	Parallel Factor Analysis
Pg	Petagram = 10^{15} g
POC	Particulate organic carbon
PT	Pressure transducer
Q	Discharge measured in cubic metres per second (m^3 s^{-1})
RSD	Relative standard deviation
SD	Standard deviation
SEPA	Scottish Environmental Protection Agency
SRP	Soluble reactive phosphorous
$SUVA_{254}$	Specific ultraviolet absorbance at 254 nm
$SUVA_{410}$	Specific ultraviolet absorbance at 410 nm
TON	Total oxidised nitrogen
WL	Whitelee
µg l^{-1}	micrograms per litre
[ ]	Square brackets are used to indicate concentration

1 Introduction

1.1 Thesis aims

The concentration of terrestrial-derived dissolved organic carbon (DOC) has been steadily increasing in many catchment drainage systems in Europe and North America (e.g., Lapierre et al., 2013; Larsen et al., 2011; Monteith et al., 2007). Land use is recognised to influence carbon's functioning in freshwater biogeochemical cycles (Noacco et al., 2017; Williams et al., 2010; Worrall et al., 2012), but the focus has generally been on DOC concentration and not composition, despite the links between DOC composition and degradation processes (Hosen et al., 2014; Kalbitz et al., 2003a). Fewer studies have explored how DOC quality changes over time and spatially, and if the increased aquatic DOC exported is resistant to in-stream degradation processes, or labile and used easily. A recent land use change important in the UK is the use of carbon-rich soils, peatlands, for wind farm renewable energy generation (Smith, 2016). How the amount of C exported from these sites in catchment drainage changes in response to the disturbance associated with development is still not well understood, and the composition of that C has rarely been considered.

Surface waters integrate extrinsic and intrinsic factors (such as DOC sources and quality, land use, nutrient and key element availability) which can affect DOC properties, and play a potentially important role in the global carbon cycle (Cole et al., 2007; Wollheim et al., 2015). The fate of DOC in aquatic systems is still poorly constrained, although there is an increasing recognition that some terrestrial DOC can be quite bio-reactive (e.g. McCallister and del Giorgio, 2012). Exploring this further, particularly in peatland drainage systems - globally important terrestrial C reservoirs that export C to drainage systems - is needed to improve knowledge of how much DOC exported may reach the oceans, and so refine models of global carbon budgets.

Considering DOC export dynamics is not just important for understanding its fate and the wider C cycle. The C cycle interacts with other biogeochemical cycles, some well-known, such as nitrogen and phosphorus (Coble et al., 2016; Oviedo-Vargas et al., 2013; van Groenigen et al., 2006), and others of growing interest. For example, recently it has been observed that iron (Fe) concentration increased with increasing DOC concentration ([DOC]) in some boreal freshwaters (Knorr, 2013; Oni et al., 2013), and in turn Fe may impact on carbon and nutrient mobility and biodegradability (Emsens et al., 2016; Xiao et al., 2016). Humic substances (HS) are an important pool quantitatively in dissolved organic matter (DOM), and are strong chelate ligands for Fe binding (Krachler et al., 2010). The analysis

of Fe-DOC interaction (e.g. complexation and co-precipitation) in response to different DOC composition, could improve understanding of the quantity of DOC export.

The over-arching aim of this PhD is to fill some of the gaps in our understanding identified above, of the importance of dissolved organic carbon composition in the fluvial C cycle. This aim has two major components:

1. To understand if land use imparts a control of fluvial DOC and if so whether this is maintained over time. Specifically, this research focuses on catchments draining wind farm constructed areas as there is little understanding of this new land use.

2. To understand how that DOC may be biologically degraded with the river system, and if land use-induced composition is a control on these breakdown dynamics.

Specific research objectives in the PhD aligned with these components were to:

1. Explore how long disturbance by wind farm development affects water chemistry in peaty catchments, by analysing the long-term trends in fluvial DOC and nutrient concentrations in streams draining the wind farm.

2. Assess if different wind farm-associated land uses, that is wind farm construction and deforestation, are another control on spatial variation of fluvial DOC quantity, and importantly, DOC composition.

3. Investigate relationships between total dissolved Fe concentration and concentrations of DOC and nutrients (phosphorus and nitrate), as this interaction may ultimately shape the DOC biodegradability during fluvial transport.

4. Quantify how much DOC is biodegraded in peatland streams and what controls this.

To address these aims, it is crucial to first understand the significance of this area of research and general knowledge about DOC chemical composition. Thus in this chapter, an overview will be given of the key components of important aspects of DOC production and export relevant to the PhD research, variation of DOC composition and the methods to characterise it. The detailed literature for each aspect of research will be further introduced within the relevant chapter.

1.2 The significance of dissolved organic carbon to global carbon cycling and aquatic ecosystems

Dissolved organic matter is a central component of biogeochemical cycles and one of the largest sources of biological available organic carbon (Battin et al., 2009; Evans et al., 2005). Dissolved organic carbon (DOC) is a dominant fraction of DOM, which is generated from the partial decomposition and subsequent leaching of living organisms including plants, animals and soil microbes (Evans et al., 2005). Being a major form of terrestrial organic carbon transported by river systems (Wollheim et al., 2015), therefore there are two main sources of aquatic DOC: (1) allochthonous terrigenous origin transported laterally from land (Fig. 1-1), with the export rate determined by production and sorption rate as well the availability of water pathways through the landscape (Ledesma et al., 2015; Roulet and Moore, 2006); (2) autochthonous substances resulting from biological activities within the water body such as the breakdown of bacteria, algae and/or higher plants (Frimmel, 2005; Kowalczuk et al., 2009). These two aquatic DOC sources differ in properties such as spectrofluoremetric characteristics (Mcknight et al., 2001) and bioavailability to in-stream microbial communities (Graeber et al., 2012).

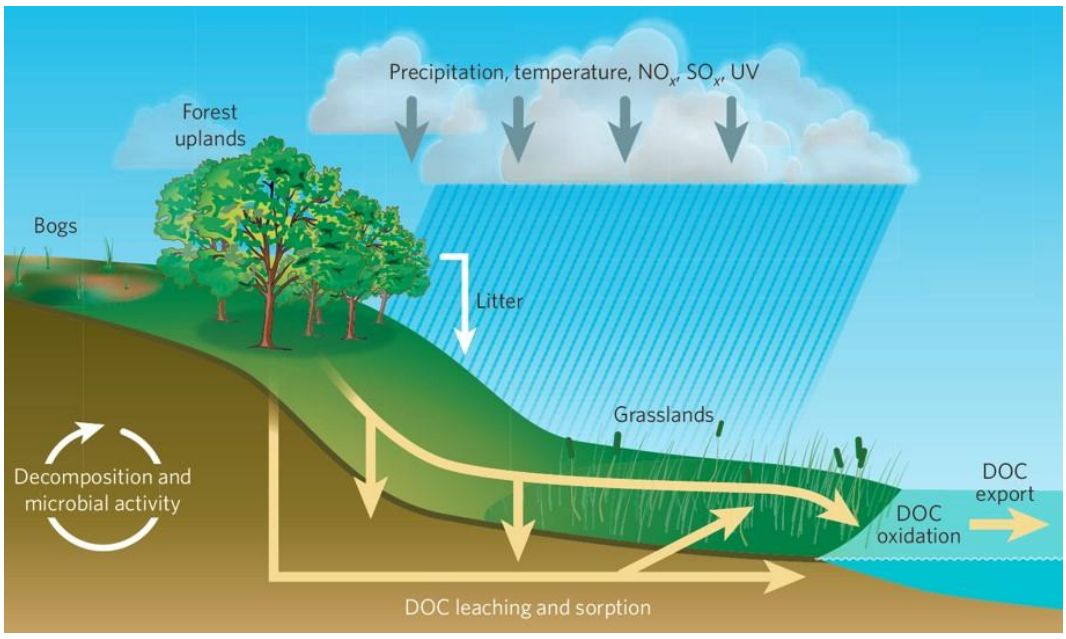


Fig. 1-1. Pathways for DOC transport through landscape (Roulet and Moore, 2006).

DOC is the most important intermediate in global carbon cycling, with the flux of terrestrial DOC (an estimate of 0.17 – 0.25 Pg C yr^{-1} from the major world rivers, Cai, 2011; Cauwet, 2002; Dai et al., 2012) being the largest transfer of reduced carbon from land to aquatic and ultimately marine ecosystems (Battin et al., 2009; Bauer et al., 2013; Roulet and Moore, 2006). Aquatic DOC can influence the functioning of freshwater

ecosystems in several ways. With a direct effect on availability and spectral quality of light in the water column, DOC largely determines optical properties of natural waters (Kowalczuk et al., 2009). By attenuating UV light, DOC shields in-stream organisms from harmful UV light (Schindler et al., 1997). The mediated changes in light regime further initiates photoreactions, which are suggested to control primary production and related trophic processes (Hansson et al., 2012; Karlsson et al., 2009). DOC is also linked with trace metal transport through complexation reactions in aquatic systems and then releasing metals to the marine environment during DOC remineralization (Kowalczuk et al., 2009; Lawlor and Tipping, 2003; Nierop et al., 2002). In addition, DOC also provides essential energy and nutrients for microbial and plant community (Wetzel, 1992), and contributes measurably to surface water acidification (Eshleman and Hemond, 1985).

1.3 The increasing trend of aquatic [DOC]

Compared to the magnitude of soil carbon loss in gaseous form e.g. carbon dioxide (CO_2) or methane (CH_4), terrestrial DOC delivery to surface waters is small, and thus was generally neglected (Billett et al., 2006). However, in recent decades it has been observed that [DOC] in surface waters has increased in much of northern and central Europe and eastern North America, with the most frequently significant increasing trends (> 0.15 mg l^{-1} yr^{-1}) observed in the Southern Nordic region and the UK (Monteith et al., 2007). But it is noted that not all waters showed the [DOC] increase. For example, significant annual [DOC] decreases were also observed in Sweden and Finland (dark blue dots in Fig. 1-2).

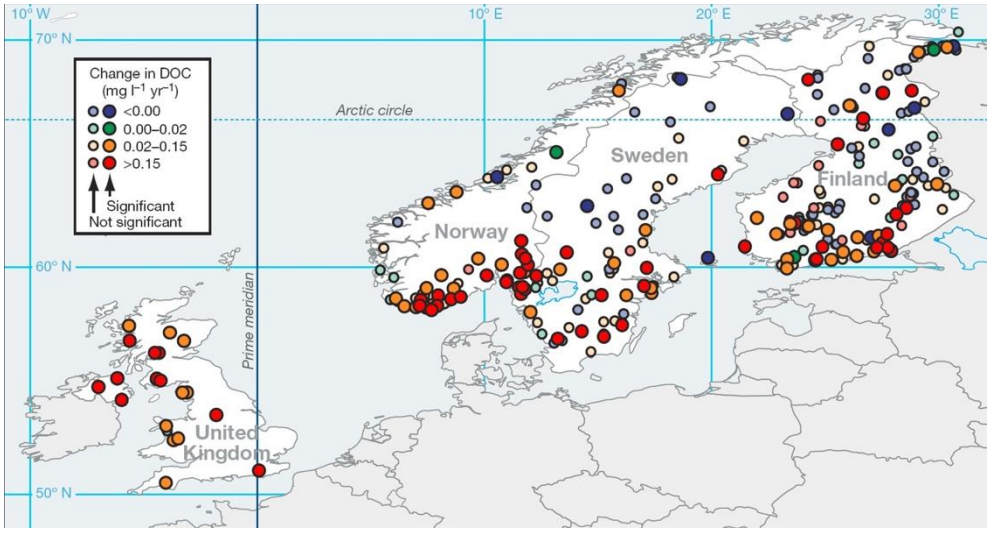


Fig. 1-2. Annual trends in DOC concentration (mg l^{-1} yr^{-1}) on acid-sensitive landscape in Europe during 1990 – 2004 (Monteith et al., 2007).

In UK, of 198 stream and lake monitoring sites with varying record lengths as far back as 1961, the average annual [DOC] increase was observed to be 0.17 mg l^{-1} yr^{-1} (Worrall et al., 2004). Further, an average 91 % increase was observed for [DOC] measured from lakes and streams in the Acid Waters Monitoring Network of the UK over the period 1988-2003 (Evans et al., 2006). This observation has since been updated for between 1988 – 2008, and significant increasing trends for [DOC] are still observed for most of the sites (Monteith et al., 2014). In 2002, a DOC flux from the UK of 0.86 Mt C was suggested, increasing at 0.02 Mt C yr^{-1} (Worrall et al., 2004). Between 2001 and 2007 an increased annual DOC flux of 0.91 Mt was estimated for 194 British catchments (Worrall et al., 2012).

Several hypotheses have been suggested to explain this widespread increase, including 1) rising temperatures as a result of global warming, increases the export of DOC from peatlands by stimulating more microbe-related DOC production (Freeman et al., 2001); 2) increased precipitation and river discharge, which are typical of the northern hemisphere where more peat carbon globally exists, leads to shorter water retention time and thus less in-lake DOC removal processes (dominated by photochemical and microbial decomposition) (Tranvik and Jansson, 2002); 3) elevated atmospheric CO_2 concentrations stimulates more primary production and DOC exudation from plants, so increasing the soil carbon pool size (Freeman et al., 2004); 4) reduced acid deposition no longer suppresses DOC production (Monteith et al., 2007), but supports increased DOC mobility and thus export (Clark et al., 2005). In addition an enzymatic 'latch' is proposed to be triggered by lowered water table and increased peat aeration during drier periods, which may release more DOC (Freeman et al., 2001). Further, this 'enzymatic latch' effect may be accentuated by local factors such as land use. Indeed, land use such as peat drainage (Worrall et al., 2003), agriculture (Graeber et al., 2012; Shang et al., 2018) and deforestation (Gandois et al., 2013) have been observed to increase [DOC] in draining waters and alter DOC export, possibly via soil erosion and decomposition, reduced soil organic matter retention and increased nutrient inputs. Heather burning is another common land use management on upland peatlands which can also increase the local fluvial [DOC]. This is suggested to be caused by increasing aerobic microbial activity in the exposed bare peat surface after burning (Yallop et al., 2010; Yallop and Clutterbuck, 2009). The increase in fluvial [DOC] highlights the importance of understanding the transfer of carbon between soil and fluvial systems, and the impacts of climatic and anthropogenic changes.

Yet agreement has not been universally accepted for the key driver of long-term [DOC] increase. However, it is also suggested that data behind these hypotheses can support each other in some way, and concluded that acid deposition may contribute to the

apparent [DOC] difference between those studies, while climate factors are more related to the seasonality (Clark et al., 2010). The impacts of sulfate deposition were further tested more recently, both in the long-term-monitored acid-sensitive surface waters and soil waters in UK (Monteith et al., 2014; Sawicka et al., 2016). It was concluded that the recovery in acidified surface water and enhanced DOC mobilization in surficial organic soil due to reductions in atmospheric sulfate deposition were the major reason leading to [DOC] increase.

1.4 Peatlands as important source for DOC and peatland degradation

Peatlands are a significant form of wetland with an organic soil layer greater than 30 cm, and may extend to 15 – 20 m depth, although the estimated mean peat depth in northern peatlands is between 1.3 – 2.3 m (Turunen et al. 2002). Peatlands are sensitive systems requiring specific climate conditions, and poor drainage to grow, develop and stabilise. Peatlands cover approximately 3 % of the Earth's land surface, 80 % of which are in the northern hemisphere, especially in America, Canada, Northern Europe and Russia, and were typically formed at 10 ka BP (10000 years ago) (Limpens et al., 2008; Yu et al., 2010).

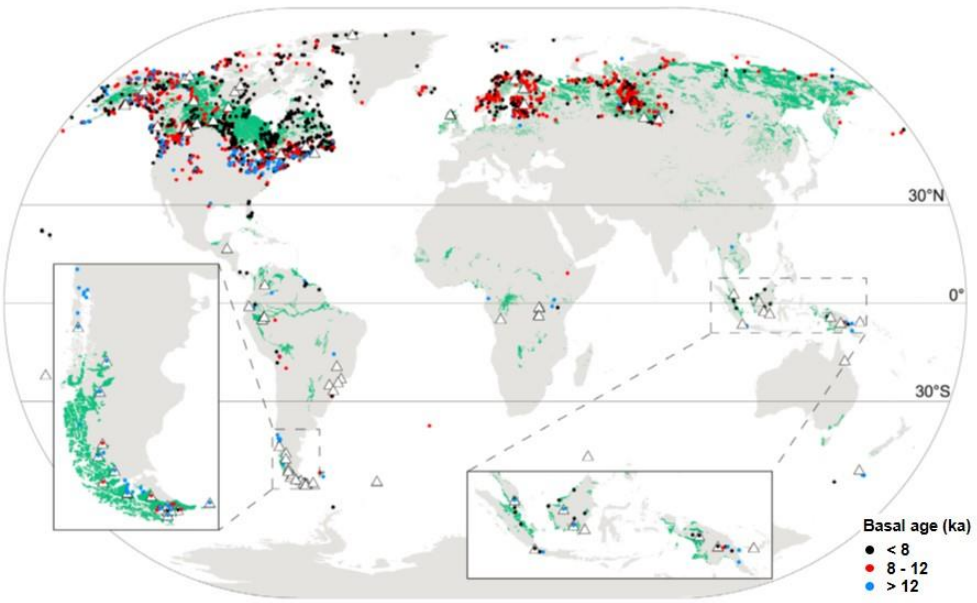


Fig. 1-3. Global peatland regions with basal peat ages where available. Large open triangles represent sites where detailed carbon accumulation rates were measured (Yu et al., 2010).

Peatlands are a vast store of terrestrial carbon, holding approximately 612 Gt soil carbon stock (approximately 40 % of global soil organic carbon) (Bridgham et al., 2006; Scharlemann et al., 2014; Yu, 2011). Among the major peatland regions, northern

peatlands are the best studied region by far. It is estimated that northern peatlands have accumulated 270 – 547 Gt peat carbon (Gorham, 1991; Turunen et al., 2002; Yu et al., 2010), representing about 90 % of the total peatland carbon pool and 30 % of the world's soil carbon stock (Gorham, 1991; Pastor et al., 2003; Yu, 2011). The estimates of C stored in tropical peatlands range from 50 – 70 Gt (Page et al., 2004; Yu et al., 2010). In the UK, an estimated 5.1 Gt carbon is stored in peatlands, of which the majority is in Scotland (4.5 Gt carbon) (Ostle et al., 2009; Smith et al., 2007). Peatlands also undertake significant gaseous carbon exchange with the atmosphere (Frolking and Roulet, 2007; Korhola et al., 2010; Roulet et al., 2007; Yu, 2011), and are a principal DOC source to the fluvial environment (Worrall et al. 2006; Roulet et al., 2007). Mean annual DOC export in local catchment-scale was estimated to range from about 7 to 60 g m^{-2} yr^{-1} in the northern peatlands, e.g. Norway(de Wit et al., 2016) Sweden (Leach et al., 2016; Ledesma et al., 2015), Ireland (Koehler et al., 2011), Canada (Roulet et al., 2007) and UK (Dinsmore et al., 2010; Evans and Maria, 2016; Niekerk, 2012).

During the last several decades, anthropogenic activities have caused peatland degradation through drainage, burning, and peat extraction for afforestation or to improve agricultural productivity (Clay et al., 2009; Holden et al., 2004; Peacock et al., 2013). Among these, artificial drainage is a significant threat to peatland sustainability (Wallage et al., 2006). In the UK, during the middle of 20th Century, around 0.5 million ha of peatlands were drained artificially and extensively for afforestation in response to government grants (Hargreaves et al., 2003; Wilson et al., 2011), and now great concern has arisen about the drainage compromising peatland functioning (Lamers et al., 2015). In recent years, an anthropogenic land use prevalent for the British peatlands (especially in Scotland) is the hosting of renewable energy developments in the form of commercial onshore wind farm (Scottish Natural Heritage, 2013). The potential impacts of such land use change on DOC quality in the drainage water is one of research aims in this PhD project. Wind farm construction and operation is associated with deforestation and road building. During construction, carbon can be lost from peat excavation (required for borrow pits, access tracks and turbine foundations), and from tree biomass due to deforestation (to ensure yield of wind energy) (Drew et al., 2013; Ostle et al., 2009). Further, preliminary research shows establishment of wind farm turbines can lead to losses of terrestrial carbon and nutrients, influencing water biogeochemistry and the ecology of the receiving streams (Grieve and Gilvear, 2008; Millidine et al., 2015; Waldron et al., 2009).

Peatlands act as a carbon sink due to the greater rate of biomass production than decomposition (Turunen et al., 2002). However, with these growing concerns about

peatland damage, and the observations of increasing [DOC] in rivers draining peatlands (Evans et al., 2002; Freeman et al., 2001), there is concern that the large amount of stored carbon is beginning to destabilize (Freeman et al., 2004; Koehler et al., 2011), with the peatlands susceptible to changing release of carbon to atmosphere or aquatic systems, further compounded by response to global warming (Limpens et al., 2008; Wu and Roulet, 2014). Indeed, totally about 30 – 50 % of the peatland annual net carbon accumulation has been found to be lost through runoff (Koehler et al., 2011; Leach et al., 2016; Nilsson et al., 2008; Olefeldt et al., 2012).

Consequently, corresponding peatland restoration activities have been carried out to preserve and re-establish peatland functions and ecosystems with some positive feedbacks (Lamers et al., 2015). For example, drain-blocking is a commonly-used peatland restoration action, suggested to reduce peat erosion, water colour and aquatic organic carbon flux (Armstrong et al., 2010; Wallage et al., 2006; Wilson et al., 2011). In the UK afforestation on land with peat over one metre deep has now been discouraged by the government (Holden et al., 2004).

1.5 The fate of DOC within river systems

While the magnitude and regulation of the increased terrestrial-derived [DOC] in inland waters has been the focus of research (e.g. Couture et al., 2012; Erlandsson et al., 2008; Noacco et al., 2017), the fate of such increased carbon input within river systems and potential implications for the aquatic ecology and carbon budgets has been insufficiently understood. Annually the worldwide river networks receive about 3 Pg of total carbon from lands (Tranvik et al., 2009), however, little terrestrial organic carbon is found in the global ocean (Bianchi, 2011), with about two thirds of land-derived carbon loss during the transport to oceans (Tranvik et al., 2009). River and stream ecosystems are considered to re-process and recycle aquatic carbon to the atmosphere as CO_2 (an estimate of 1.4 Pg yr^{-1}) and CH_4 (0.65 Pg yr^{-1}) (Bastviken et al., 2011; Battin et al., 2009). The loss of organic carbon during this passage is significant in order to better constrain carbon loss as DOC and gaseous carbon, a critical component of the global carbon cycle (Catalán et al., 2016; F Worrall et al., 2006). Therefore it is crucial to understand the stream ecosystem metabolism, which integrates production and respiration of organic matter (Fuß et al., 2017).

When terrestrial DOC is mobilized into fluvial systems, the lack of in-soil constraints on degradation (such as sorption to mineral surfaces and absence of light) reduces DOC persistence (Kellerman et al., 2015). Multiple DOC processing pathways exist in aquatic

systems, including photo- and biodegradation, sedimentation and burial within freshwaters (Cole et al., 2007; Weiser and Robarts, 2004). Among these processes, photo- and biodegradation are simultaneously active and suggested to account for the most of organic carbon loss in gaseous form (CO_2 and CH_4) from surface waters (Catalán et al., 2016). In addition, the mineralization and removal of DOC can further affect in-stream food webs as terrestrial organic matter predominantly fuels the primary and bacterial production in waters, and support higher trophic level biomass together with autochthonous organic matter (Karlsson et al., 2012).

Landscape and climate controls on fluvial DOC quality and in-stream fate have been explored. The results from a 560 boreal lake survey suggest land cover, especially the percentage of water in the catchment (thus duration of time taken to pass through the landscape), is a more important factor than mean annual temperature in influencing the variability in DOM composition (indicated by fluorescent components) (Kothawala et al., 2014). More persistent DOC remains after fluvial transport. This is consistent with the observation of a negative relationship between water retention time and organic carbon degradation rate across a wide range of aquatic systems (Catalán et al., 2016), with longer time providing more chance for in-stream organic carbon processing.

Further, as land is the major source for aquatic DOC, land use has been increasingly recognised as a significant influencing factor mediating the composition and thus degradability of DOM via biogeochemical and biodegradation processes in aquatic networks (e.g. Butman et al., 2014; Lu et al., 2013; Williams et al., 2010). For example, human land uses (including agriculture, pasture and urban area) have been suggested to decrease the proportion of terrestrially-derived humic organic matter and increase the proportion of autochthonous sources in the draining water (Hosen et al., 2014; Lu et al., 2014; Shang et al., 2018; Williams et al., 2010). This may be caused by the modification of terrestrial landscape (e.g. anthropogenic land use-induced erosion and oxidation), leading to the increased microbial activity in human-affected streams (Lu et al., 2014; Williams et al., 2010).

The diversity of DOC molecules can lead to the considerable temporal and spatial variation in estimates of carbon removal rates (Wollheim et al., 2015). Intrinsic molecular structure has been suggested to be a more important control on DOC stability in streams than extrinsic factors (such as environmental conditions), with the decomposition (biodegradation and photoreaction) occurring along a gradient from aromatic to aliphatic compounds (Kellerman et al., 2015). Further, compositional difference has also been considered to influence the varied responses of autochthonous and allochthonous fluvial

DOC to biodegradation and photoreaction. Autochthonous DOC typically contains smaller molecular weight compounds and few aromatic ring structures, and thus is suggested to be highly biodegradable (Barrón et al., 2012; Wickland et al., 2007), while allochthonous DOC is generally more recalcitrant (Graeber et al., 2012). Therefore the estimate of carbon loss via biodegradation of terrestrial DOC is modest (Lapierre et al., 2013).

However this suggestion is being challenged as more evidence shows terrigenous humic DOC can be quickly respired by microorganisms in fluvial ecosystems (Berggren et al., 2010; Fasching et al., 2014; McCallister and del Giorgio, 2012). This emphasizes the need to better explore this pathway of carbon loss and the importance of considering inherent DOC properties in this process. While fresh and less-humic DOC is commonly considered to be labile to microbes, photo-degradation is mainly considered to affect coloured, photo-reactive carbon with high aromaticity and oxidation state (Kellerman et al., 2015; Lu et al., 2013; Stubbins et al., 2010). This is possibly because the formation of complexes between humic acids and fulvic acids (main fractions of DOC) and extracellular enzymes inhibits enzyme activity and prevents biological DOC degradation, but can be decoupled by mild ultraviolet photolysis (Wetzel, 1992).

1.6 Variation of DOC composition and its significance

As the reactivity, decomposition, and thus fate of DOC is closely and inherently linked to its chemical composition and molecular properties (Kellerman et al., 2015; Kothawala et al., 2014; Sun et al., 1997), it is crucial to characterise and understand DOC composition. However it is difficult to provide a chemical description of DOC in waters, as it changes as a function of hydrology and climate (Kothawala et al., 2014). In general DOC consists of a variety of molecules considered to range in size and structure, from simple identifiable, low-molecular weight compounds such as acids and sugars, to complex, high-molecular weight humic substances (HS) (Thurman, 1985).

Non-humic substances are more easily consumed by microorganisms and invertebrates, and thus have a shorter survival rate compared to the more biologically-resistant HS (Khan and Schnitzer, 1972; Kulovaara et al., 1996; Thurman, 1985). In addition peat soil organisms, principally fungi and bacteria, metabolize the non-coloured, small molecular mass non-humic substances in preference to the larger coloured HS (Thurman 1985; Hope et al. 1994; Dawson et al. 2001).

Traditionally, HS are thought to comprise the dominant fractions of DOC, and are heterogeneous mixtures formed by humification, the biochemical and chemical reactions

that occur during the decay and transformation of plant and microbial remains (Muscolo et al., 2013). HS are a complex mixture of aromatic and aliphatic hydrocarbon structures with attached amide, carboxyl, ketone and other functional groups (Leenheer and Croue, 2003). HS have never been separated into pure components (Hautala et al., 2000; Muscolo et al., 2013), but can be generally divided into two main fractions based on chemical extraction processes: humic acids (HAs) and fulvic acids (FAs) (International Humic Substances Society; Frimmel, 2005; Muscolo et al., 2013). HAs are categorized as the fraction that is not soluble in water under acidic conditions (pH < 2) but are soluble at higher pH values. Generally HAs have a larger molecular weight, contain more carbon with less oxygen-containing functional groups (Stevenson, 1994; Weng et al., 2006; Gungor and Bekbolet, 2010; Tang et al., 2014). They have a greater proportion of hydrophobic (mainly aromatic) moieties (Piccolo, 2001; Šmejkalová and Piccolo, 2008), are more stable as they have more conjugated bond systems (formed by several multiple bonds, each separated by single bonds). Therefore, HAs are more complex and microbially-resistant than FAs. The structural difference suggests HAs to be more humified and aromatic than FAs.

Clearly the composition of DOC will influence how easily it can be degraded biologically and so methods are required that allow compositional controls to be understood. The bond structure and molecular size properties allow the use of spectrophotometric methods to consider structural characteristics: aquatic DOM strongly absorbs light in the UV-visible wavelength range, with compositional differences influencing absorbance at specific wavelengths (Korshin et al., 1997; Helms et al., 2008; Selberg et al., 2011; Peacock et al., 2014).

1.7 Characterising DOM quality through UV-visible and fluorescence spectroscopy

UV-visible spectroscopy

Chromophoric dissolved organic matter (CDOM) is the fraction of DOM which is optically active and absorbs both visible and UV light (Helms et al., 2008). With the compositional differences between DOC fractions influencing absorbance at specific wavelengths, these DOM properties allow the use of spectrophotometric methods to consider structural characteristics (Korshin et al., 1997; Helms et al., 2008; Selberg et al., 2011; Peacock et al., 2014).

Specific UV absorbance at 254 nm ($SUVA_{254}$) is one of the commonly used optical parameters, measured by dividing the absorbance of a water sample at 254 nm by its DOC concentration. $SUVA_{254}$ is considered to be strongly related to DOC aromaticity (Weishaar et al. 2003), especially the proportion of the total carbon in aromatic rings (Zbytniewski and Buszewski, 2005). Although non-humic substances are generally not aromatic, the amino acids phenylalanine, tryptophan and tyrosine do have aromatic rings and UV absorption peaks in the region 200 - 300 nm. Therefore $SUVA_{254}$ represents all aromatic carbon. In addition, HS with the more-complex aromatic structure absorb visible light mainly at the end of blue spectrum (about 400 nm) (Evans et al., 2005). As a result, $SUVA_{410}$ (absorbance at 410 nm divided by [DOC]) has been used to reveal the changes in proportion of HS which contain complex aromatic structure.

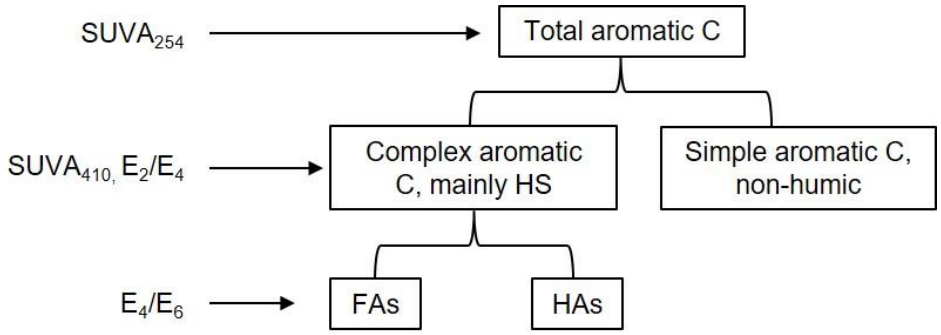


Fig. 1-4. The relationship between total aromatic C, simple aromatic C, HS, HAs and FAs. The four optical parameters can be used to indicate the composition of DOC.

The ratios between absorbance at different wavelengths can also provide qualitative information about DOC composition. The ratio between absorbance at 254 nm and 410 nm (E_2/E_4) is related to UV-absorbing aromatic rings to HS colour (Graham et al., 2012; Selberg et al., 2011; Zepp and Schlotzhauer, 1981). Thus waters which contain a greater proportion of HS have lower E_2/E_4 values (Graham et al., 2012). In addition, as HAs and FAs absorb light in different amounts at 465nm and 665nm, the ratio between absorbance at 465 nm and 665 nm, E_4/E_6, is used to reveal differences in the proportion of humic and fulvic acids (Thurman 1985; Hautala et al., 2000; Spencer et al. 2007; Moody et al. 2013). A lower E_4/E_6 reflects a larger proportion of HAs, a higher degree of aromatic condensation and indicates a higher level of organic material humification (Zbytniewski and Buszewski, 2005). Between aquatic humic and fulvic acid samples, E_4/E_6 has been observed to be higher for fulvic acids and lower for humic acids (Thurman, 1985).

Fluorescence spectroscopy

A sub-fraction of CDOM fluoresces and is termed FDOM (Coble et al., 1990; Stubbins et al., 2014). In recent years there has been a growing interest in the use of fluorescence spectroscopy to characterise the complex DOM chemical composition (e.g. Coble et al., 1990; Cory and Mcknight, 2005; Fellman et al., 2010). The fluorescence excitation-emission matrix (EEM) analysis, in which repeated emission scans are collected at numerous excitation wavelengths, and Parallel Factor Analysis (PARAFAC), a three-way statistical modelling technique, have been combined and developed to decompose the overlapped fluorescent signal of DOM into broadly identified individual components (Bro, 1997; Cory and Mcknight, 2005; Stedmon et al., 2003). The most-commonly observed fluorophores consist of several humic-like (carbon associated with HS) and protein-like components (carbon associated with fluorescent amino acids), ranging in molecular weight and derived from autochthonous production, microbial processing and terrestrial systems (Cory and Kaplan, 2012; Fellman et al., 2010; Maie et al., 2007).

PARAFAC has been used to characterize DOM and assess its dynamics in fluvial systems, providing insights into the different biological lability of these various types of organic carbon pools. Among these fluorophores, protein-like DOM fluorophore is suggested to represent a product of bacterial activity and a bioavailable substrate (Cammack et al., 2004), with the proportion of protein-like components often being linked to DOM biodegradability across different environments (e.g. Balcarczyk et al., 2009; Fellman et al., 2009). However, a recent study further suggests the protein-like fluorophore comprises not only labile DOM, but also semi-labile and more recalcitrant moieties (Cory and Kaplan, 2012). In addition, the humic-like fluorophores are generally considered to be aromatic with hydrophobic components, and used as a proxy for slowly cycling and recalcitrant DOM to biodegradation (Fellman et al., 2010). With such understanding, PARAFAC modelling has been used to characterize aquatic DOM quality in different environments (e.g. Beggs and Summers, 2011; Osburn et al., 2012; Zhang et al., 2011), and to explore the relevant biogeochemical significance, including landscape and land use influence on DOM biodegradability. For example, catchment urbanization were found to be associated with increased protein-like DOM and decreased amount of terrestrial humic-like DOM (Hosen et al., 2014), indicating a likely increased in-stream DOM biodegradation.

1.8 Summary of the literature review

From the above literature review, key understanding can be summarised as follows:

DOC is an essential component of the global carbon cycling and can have important ecological impacts on aquatic systems and surface water quality. Peatlands, as a significant terrestrial DOC source, are associated with the widely-observed [DOC] increase in surface waters. Anthropogenic land use change in peatlands is suggested to be an influential factor, along with climatic change, discharge increase, and acid deposition decrease. After export from soils, DOC undergoes multiple in-stream processes, including biodegradation, and this influences variation in both DOC quantity and quality that reaches in the oceans. The difference in DOC quality in turn is closely linked to its reactivity, degradability and fate. Yet there is still a knowledge gap in understanding of DOC quality and what controls this. With the availability of UV-visible and fluorescence spectroscopy to characterise DOM quality, produced better understanding can be generated to explore the link between fluvial DOC compositional changes and peatland land uses.

1.9 Thesis contents

This thesis comprises the following research, which address the thesis aims in Section 1.1:

Chapter 4, 'Impacts of wind farm development on spatial and temporal variation of DOC quantity and quality', examines the spatial and temporal variation in fluvial DOC concentration and optical properties in short- and long-term monitoring sites, to assess disturbance of wind farm-associated land uses to peat and adjacent water quality, and potential controls on DOC composition (Objectives 1 and 2).

Chapter 5, 'Dissolved [Fe] in peatland catchments and its effect on the biogeochemical cycles of carbon and nutrients', analyses relationships between concentrations of DOC, nutrients and total dissolved Fe in peatland catchments. The DOC composition is considered to assess if there are controls on the variation of Fe concentration in streams (Objective 3).

Chapter 6, 'Biodegradation of compositionally-different DOC from Drumtee', presents the results of DOC incubations with stream water collected over one year, to comprehensively consider the potential of DOC loss by biodegradation in peaty waters. DOC composition changes are analysed during incubation to understand DOC dynamics (Aim 4). Controlling factors on DOC biodegradation loss are explored between DOC composition and nutrients availability (Objective 4).

To support this, a general method chapter, Chapter 2, 'Methods', introduces the field sites and documents field and laboratory methods, and data processing common to all sub-projects in this research. Methods specific to a chapter are introduced in the chapter methodology. Chapter 3, 'Method developments', offers methodological information but is given separately to chapter 3 as this is primary research documenting method development for better spectrophotometric measurements and undertaken to general the best protocols to quantify DOC biodegradation in laboratory incubations. Finally, Chapter 7, 'Conclusions', returns to the over-arching objective, and brings together the key findings of this research, from which research gaps can also be identified.

2 Methods

This chapter presents a description of field sites studied in this research, comprising a small catchment as the main and intensive study field to explore DOM composition and dynamics in peaty catchment waters, and five adjacent catchments as part of a long-term-conducted water chemistry monitoring programme. The chapter also outlines the methods applied to the subsequent chapters, comprising most field methods, laboratory analysis and data processing. Significant method development is detailed in chapter 3 to differentiate it from methods already developed that I used routinely.

2.1 Field sites

2.1.1 Whitelee Wind Farm

Whitelee wind farm, in central Scotland, approximately 16 km south of Glasgow, is Europe's second largest and the UK's largest onshore wind farm. Whitelee is located mostly on peat-rich soils, with an average peat depth of 190 cm ± 134 cm (ranging from 5 cm to over 8 m, Fig. 2-1 shows the soil map for the south of the Whitelee Catchment). Peat is defined as soil where the uppermost soil horizon has more than 60 % organic matter and exceeds 50 cm in thickness (Soil Survey in Scotland, 1984; SNH 2014). Apart from peat, the area is covered by poorly drained non-calcareous gleys, peaty podzols which are freely draining below the iron pan, freely draining brown earth forest soils, imperfectly draining brown forest soils, very poorly drained humic gleys and poorly drained peaty gleys (Murray, 2012).

Wind farm construction activities took place from October 2006 and the original 140 turbines became fully operational in May 2009. In Nov. 2010, the site was extended with a further 75 turbines, completed in Feb. 2013. The wind farm is built largely in the Whitelee Forest of Sitka spruce, which was planted during the1960s – 1980s. Felling was carried out in the Whitelee Forest to facilitate wind farm construction of the tracks, turbine bases, substation, and cable installation, and to create open ground areas and thereby reduce wind turbulence for the windfarm operation. For the wind farm construction, a total of 1200 ha forest had been felled (Phin, 2015). In addition, peat excavation was required for access road construction (thus drainage), turbine bases and associated infrastructure erection as part of the wind farm development.

Table 2-1 Information about each long-term-monitored catchment draining the Whitelee Wind Farm. Information is gathered and modified from Murray (2012), Phin (2015) and Digimap® Land Cover Map 2015.

Catchment	Catchment size (km^2)	Flow Length Upstream (km)	Peat-related soils (%)	Deforested area (%)	No. of Turbines	Turbine density (per km^2)
WL13 (Drumtee)	5.7	5.3	88.0	30.3	7	1.2
WL14	15.1	8.1	78.6	10.3	38	2.5
WL15	11.3	6.0	90.0	33.6	38	3.4
WL16	30.5	10.8	82.0	13.2	52	1.7
WL1	31.1	11.4	62.0	10.7	54	1.7

Since July 2006 stream water chemistry monitoring had been conducted at the outlets of five catchments draining the south of the original and the most of expanded wind farm areas: WL13 which was also named Drumtee, WL14, WL15, WL16 and WL1 (Fig. 2-1). These catchments differ in size, with Drumtee being the smallest (5.7 km^2) and WL1 the largest (31.1 km^2) (Table 2-1, Fig. 2-1). All catchments are largely covered by peat-related soils (including peat, peaty gley and peaty podzol), ranging from 62 % in WL1 to 90 % in WL15. 189 of the 215 turbines are within these 5 catchments and 15.4 % of total catchment area was felled during wind farm development periods. Drumtee and WL15 are the two catchments with the greatest percentage of deforestation area (30.3 and 33.6 % respectively). During the long-term water monitoring, chemical analysis has been focussed on the concentration and export dynamics of organic carbon in dissolved and particulate form (DOC and POC), nitrate and phosphorus (SRP and total P) (Murray, 2012; Phin, 2015; Waldron et al., 2009). The long-term sampling points are shown in Fig. 2-1.

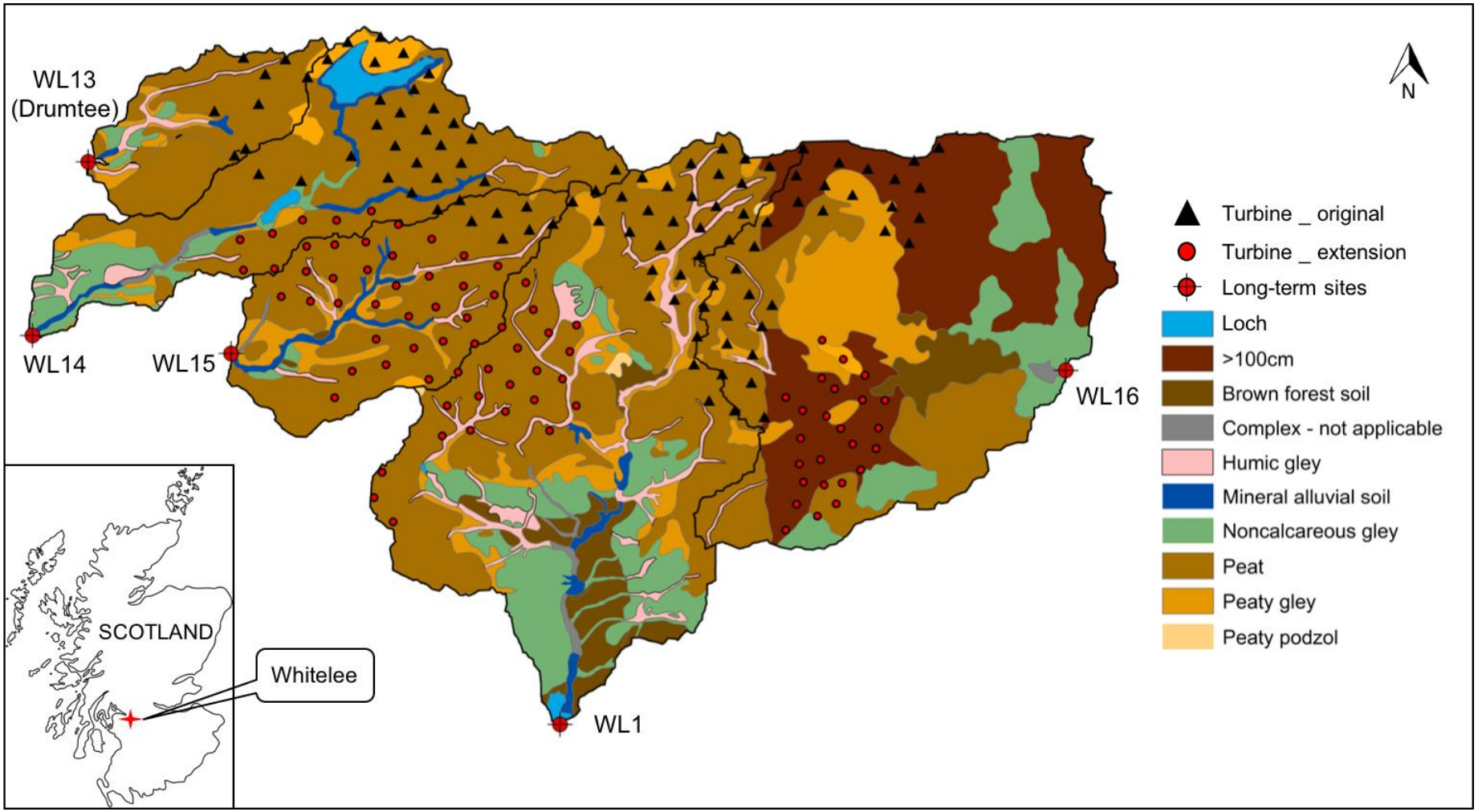


Fig. 2-1. The five long-term monitoring sites and their catchment areas. Turbines located in the catchments are shown, constructed during the original (Oct. 2006 – May 2009) wind farm development and the extension (Nov. 2010 – Feb. 2013). The soils covering these catchments are largely peat or peat-related. Soil data courtesy of Macaulay Land Use Research Institute. Turbine locations were positioned from Digimap® Land Cover Map 2015.

2.1.2 Drumtee Catchment

Drumtee (55°41'16''N, 4°23'37''W), is a third order 5.7 km^2 catchment, draining part of the western edge of Whitelee wind farm. It was chosen as a short-term field site for the studying DOC compositional changes due to its manageable size and land use difference. It has also been a site of previous research to build on (Murray, 2012; Phin, 2015; Waldron et al., 2009). The maximum elevation within the catchment is 260 m above sea level, and the catchment gently slopes downwards from the northeast to the southwest, with slopes mainly between 0 to 6 degree (Fig. 2-2). The bedrock is largely sandstone and lava, with no carbonates. The meteorological information about Drumtee is summarized in Table 2-2, indicating that the climate of the field site is normally cool and moist.

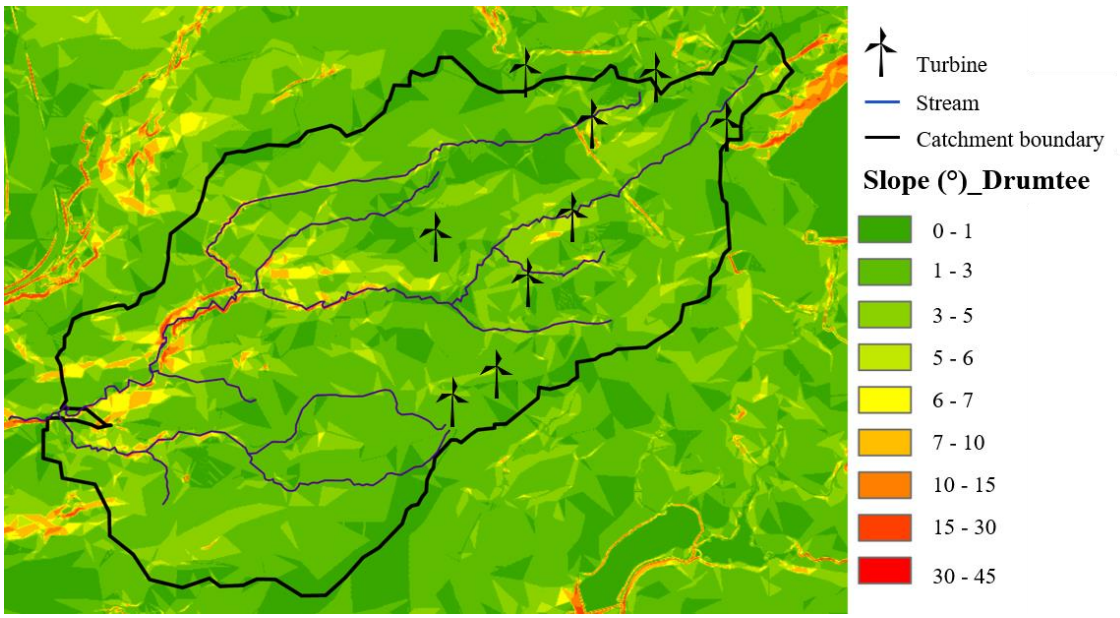


Fig. 2-2. Slope of Drumtee catchment. Data was from Digimap® OS Terrain 5 DTM.

Table 2-2 The meteorological information about Drumtee catchment (Met office, 2016)

Meteorological Data	
Climate	Temperate
Mean annual rainfall	1250 – 1500 mm
Mean annual temperature	8 – 9 °C
Mean days of snow lying (1981 – 2010)	10 - 20

There are two sub-catchments in Drumtee: D-WF in the north (3.9 km^2) and D-FF in the south (1.8 km^2). The soils in Drumtee Catchment are mainly blanket peat (Fig. 2-3A), comprising 73.5 % of the area of D-WF and 87.7 % of D-FF (Table 2-3). A further 8 % of each catchment is peaty gley area, and the remainder is composed of humic gleys, mineral alluvial soils, non–calcareous gleys and peaty podzols.

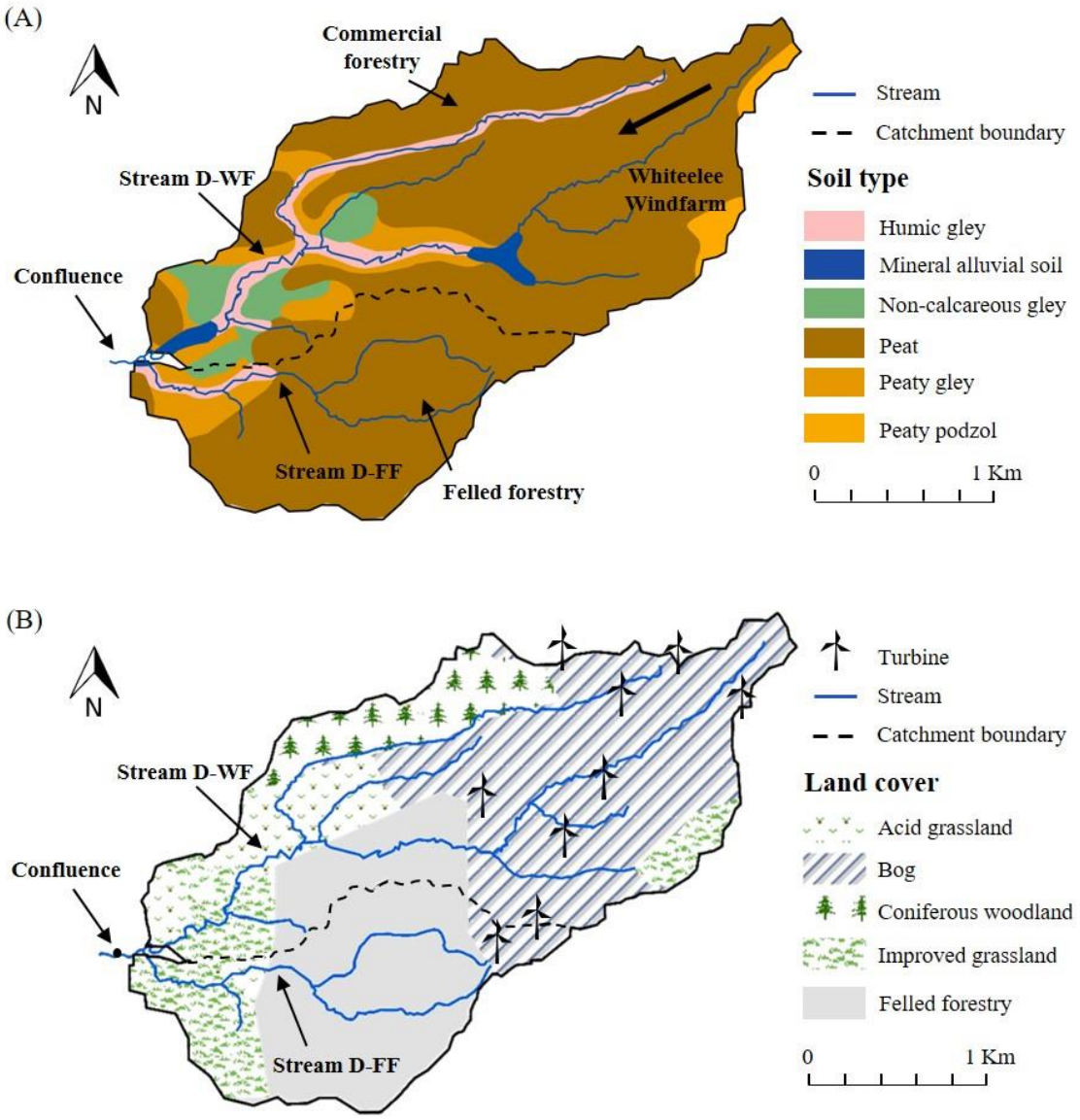


Fig. 2-3. Map of Drumtee catchment indicating different soil types (A) and land uses (B). Drumtee is draining part of the western edge of Whitelee Wind Farm, and consists of two sub-catchments. Each sub-catchment contains one main stream (D-WF in the north and D-FF in the south). There is a similar dominance of peat coverage in each catchment, but land uses differ, with D-FF dominated by deforestation whilst D-WF hosts more turbines. There are six turbines in D-WF (another one very close so could have impacted the drainage), whilst two in D-FF. One small tributary from Stream D-WF originates in felled forestry. Land use data was from Digimap® Land Cover Map 2015.

Table 2-3 Types of soils in Drumtee sub-catchments. There is a similar dominance of peat coverage in both D-WF and D-FF.

Land use	Peat	Peaty gley	Peaty podzol	Humic gley	Non-calcareous gley	Mineral alluvial
D-WF	73.5 %	8.4 %	2.6 %	7.4 %	6.2 %	1.9 %
D-FF	87.7 %	8.2 %	0	3.1 %	0.8 %	0.2 %

The vegetation in Drumtee catchment observed during field trips was mainly grass and in the more boggy areas rushes and Sphagnum moss. The land use is rough grazing of sheep

and cattle, and commercial forestry. The stream D-WF in the north sub-catchment mainly drains an area with six turbines in the headwater and intact commercial forestry, although one tributary originates in the felled area and another transits through it (Fig. 2-3B). The stream D-FF in the south sub-catchment mainly drains felled forest with only two turbines (Fig. 2-3B).

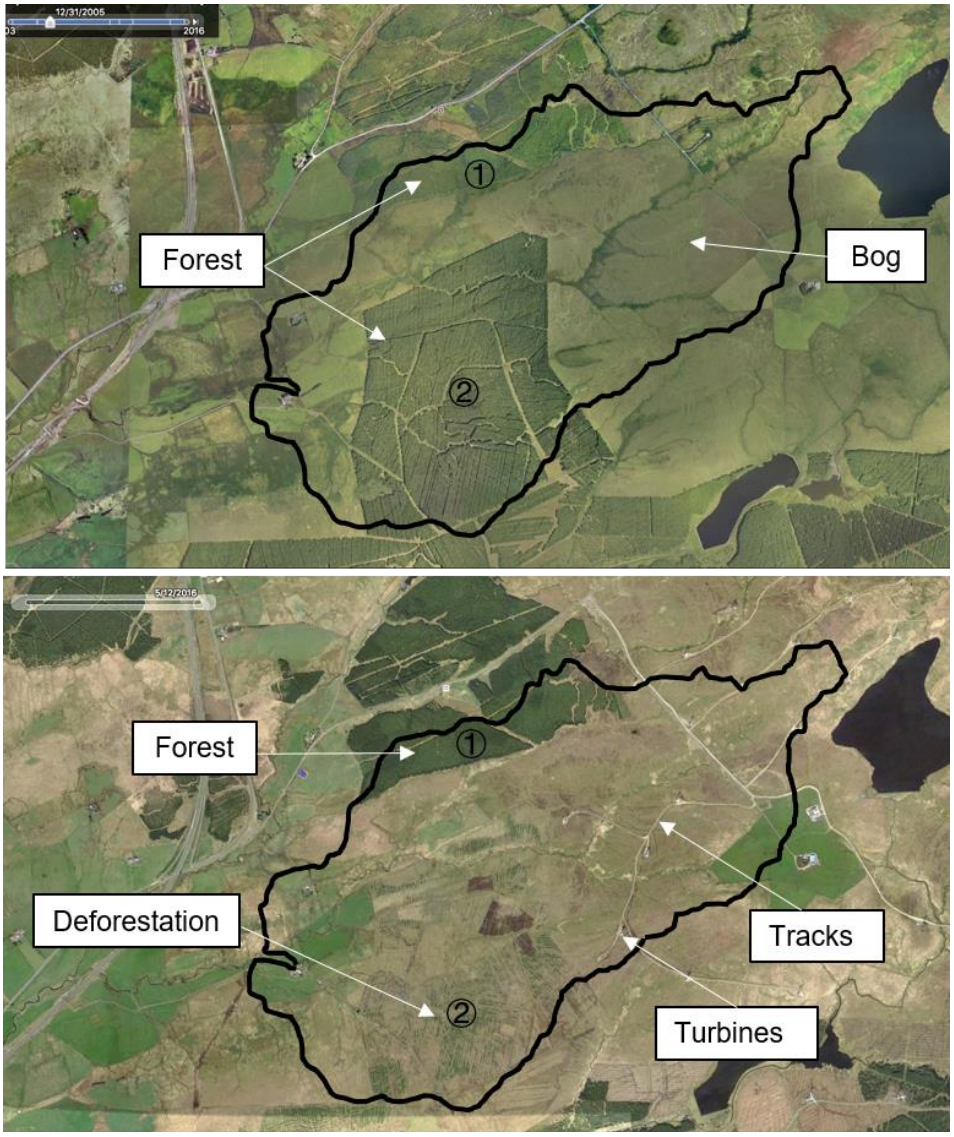


Fig. 2-4. Satellite images of Drumtee catchment on 31/12/2005 (upper) and 22/08/2017 (lower), indicating wind farm-related land use changes. Areas ① and ② were both covered with commercial forest in 2005, and due to wind farm development area ② was felled afterwards. Dark green area with slightly black lines represents commercial forest (upper), while the grey colour with lines is the felled area. Road tracks are shown by white lines. Turbines are located at the end of roads. Images were downloaded from Google™ Earth.

The original commercial forestry in both sub-catchments was planted during the 1960s – 1980s, as a part of government's afforestation plan across the country. The deforestation in Drumtee occurred during Nov. 2006 – July 2008, which was required to improve airflow and reduce wind turbulence for wind farm operation (Murray, 2012). This resulted in 70.9 %

felled area in D-FF and 12.2 % in D-WF. Road tracks and wind farm turbines were built in the upland bog area, which was not afforested historically. Two Google™ Earth images taken over Drumtee clearly showed the land use changes before and after the wind farm development (Fig. 2-4). Taken all together, the similar soil types and topography, but different land uses between the two sub-catchments provides the potential to compare impacts on water chemistry derived from land use difference within the small area.

2.2 Field methods

2.2.1 Stream water sampling procedure

As Drumtee catchment was the core field site for this research, more frequent stream water sampling was carried out here for the following three chapters: investigations in peatland catchment water for land use impacts on DOC concentration and composition (presented in Chapter 4), in-stream total dissolved Fe concentration (Chapter 5), and biodegradation of compositionally-different DOC (Chapter 6). In addition, at the outlet sites of the five south-draining Whitelee catchments (including Drumtee which was also named WL13), water was sampled and measured for concentrations of DOC, total oxidised nitrogen (TON) and soluble reactive phosphorus (SRP). This was to continue the long-term water chemistry survey which started in July 2006, from before wind farm development commenced. In this study, the long-term survey was expanded to include analysis for DOC composition and total dissolved Fe concentration. Water sampling strategy for these projects is presented as below:

Table 2-4 Sampling strategy for different projects in this study. Projects are listed according to their order in the thesis.

Project	Chapter	Sampling site	Sampling length	Sampling frequency
Continuance of long-term water chemistry monitoring (DOC, SRP, TON)	4	Whitelee catchments	Apr. 2014 - Sept. 2016	Monthly
land use impacts on DOC concentration and composition	4	Drumtee	Feb. 2014 - Feb. 2015	Monthly
		Whitelee catchments	Apr. 2014 - Sept. 2016	
Total dissolved [Fe] in water and how it impacts peatland DOC fate	5	Drumtee	Feb. 2014 - Feb. 2015	Monthly
		Whitelee catchments	Apr. 2014 - Sept. 2016	

Biodegradation of compositionally-different DOC	6	Drumtee	Sept., Nov. 2015, Jan., May 2016	Once in each sampling month

The sampling sites for different projects are indicated in Fig. 2-5. In D-WF, no sampling sites were chosen further in the bog area for two reasons: 1) the downstream sites (D-WF 5, 6, 7 and 8) received water from the upstream and integrated the potential impacts from the bog area; 2) workload and access to the bog (which hosted the turbines) were considered for a more logical sampling plan. For the short-term water chemistry investigation in Drumtee catchment during Feb. 2014 – Feb. 2015 (including analysis of DOC concentration and composition, and total dissolved Fe concentration), eight sites were sampled along Stream D-WF and seven sites along Stream D-FF approximately every four weeks (Fig. 2-5, upper). The yearly sampling allowed the annual analysis for water chemistry. For DOC biodegradation experiments, water was collected from sites D-WF1, D-FF1 and Drumtee confluence in Sept. (autumn), Nov. (early winter) 2015 and Jan. (late winter), May (summer) 2016 respectively (Fig. 2-5, upper) to analyse the seasonal and spatial variation in DOC biodegradation.

For long-term water monitoring project, stream water samples were collected from the outlet of each Whitelee catchment for a longer period: from April 2014 to September 2016 at around four-week intervals. In Drumtee, the confluence water was collected for the outlet sample WL13.

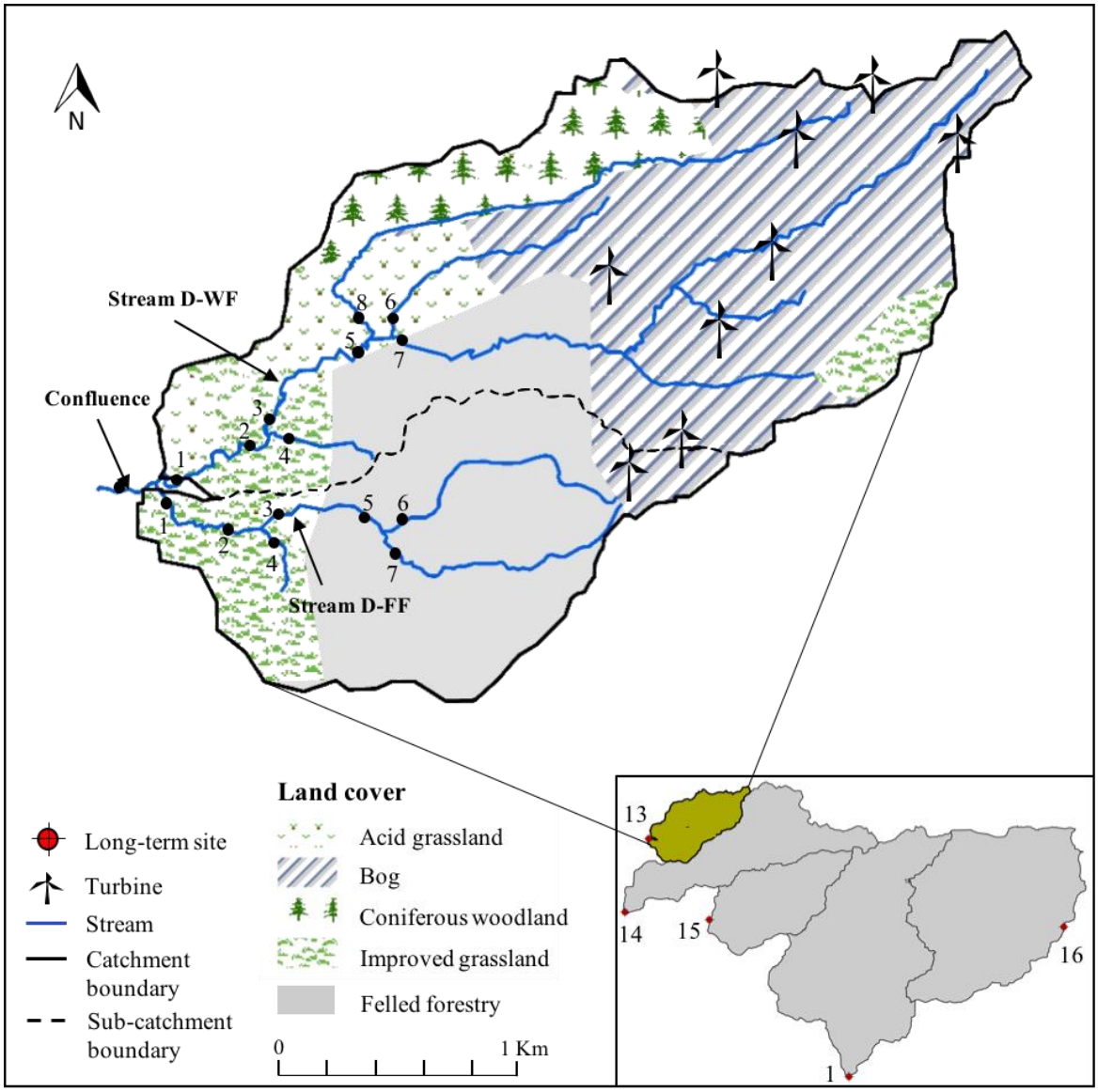


Fig. 2-5. Sampling sites in both Drumtee (upper) and other Whitelee catchments (lower right). In Drumtee, the confluence water was collected as the outlet sample for the long-term survey. D-WF represents the stream draining the wind farm area in Drumtee, and D-FF the felled forest.

Water samples for DOC and Fe analysis were collected using 330 ml drinking water bottles (PETE), which of many bottles tested had negligible release of UV-absorbance material (details demonstrated in Chapter 3 Method development). Additionally 50 ml polypropylene tubs were filled with water samples from each of long term sites for [SRP] and [TON] measurements. To remove potential contaminants all containers were pre-rinsed with deionized (DI) water in the lab and with stream water in the field three times respectively before using. Water was collected from about 5 – 10 cm beneath the stream surface and samples kept in a cool box until return to the laboratory, where after they were stored in the cold room at 4 °C until further processing.

2.2.2 Discharge measurement

Stream discharge data for Drumtee catchment provides important information to 1) analyse if there is any hydrological impact on DOC composition and concentration; and 2) estimate DOC and Fe exports (flux per year or month for each catchment area).

At the Drumtee confluence site, discharge was measured from the beginning of sampling in this project (Feb. 2014) to April 2016 using three different sensors, including a Teledyne ISCO flow logger, an In-situ Inc pressure transducer (PT) and an In-situ Inc TROLL®, which were capable of measuring water pressure and/or stream velocity, but at different time periods (Fig. 2-6). The ISCO flow logger estimated directly discharge, while both PT and the TROLL recorded stage height in the steam and discharge was calculated from the relationship with the ISCO. All sensors operated measurements at 15 minute intervals. Field trips were arranged every 2 – 3 weeks to download data, and check the battery, spectrum and signal strength to make sure the sensors were functioning properly.

The sensors were first operated and maintained by another PhD project (Coleman, 2017), and it is from his work the stage-discharge profiles (Table 2-5) have been calculated. I supplemented the discharge time series by maintaining the sensors from July 2015 to April 2016. The measurements had to be stopped to avoid potential damage to discharge sensors from a newly started construction programme at the site. In addition to the Drumtee flow data, discharge on the River Irvine was monitored at the Newmilns gauging station (station reference: NS 53252 37188) by the Scottish Environment Protection Agency (SEPA), which is the nearest gauging station to Drumtee. The accessed SEPA data was measured at 15 minute intervals and available from Aug. 2011 to Mar. 2015.

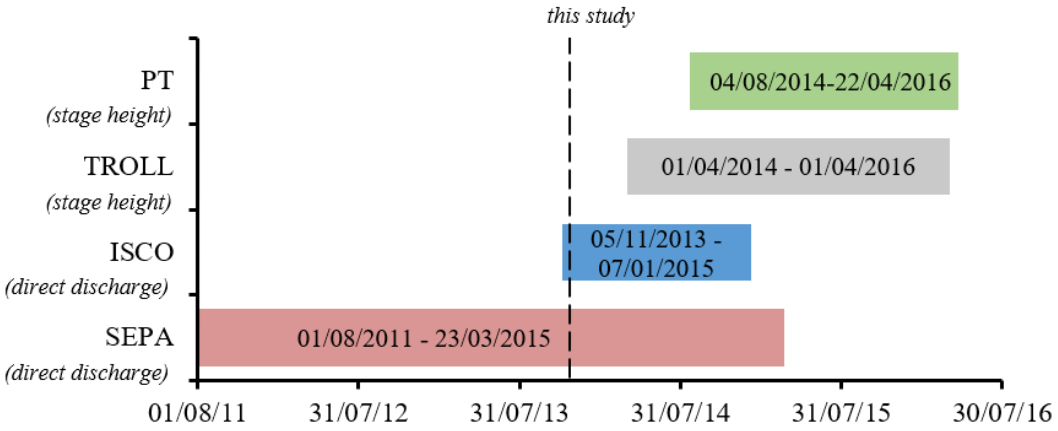


Fig. 2-6. The data sources with which Drumtee discharge can be estimated, and the different time periods when these data were available. PT and TROLL measured the stage height, while ISCO sensor and SEPA monitoring station provided direct discharge data. The dashed line shows the beginning of sampling in this PhD study (Feb. 2014).

The direct discharge data from the ISCO flow logger did not cover the full period of this study due to equipment failure in some months. When this happened, discharge was calculated with data from other sensors or the SEPA gauging data, according to their relationships with the ISCO data. The relationship between the ISCO discharge and the PT stage height data was the strongest (R^2 = 0.92) compared to those between the ISCO discharge and SEPA gauging data (R^2 = 0.89), and the TROLL® stage height data (R^2 = 0.50) (Coleman, 2017). Therefore, the time series of estimated discharge in Drumtee was constructed for 2011 – 2016. The ISCO logger data was used where available, and the second and third choices would be calculating the discharge from the PT stage height and the SEPA gauging data at Newmilns according to the equations as below (Table 2-5). The TROLL® stage height showed the weakest relationship with the ISCO discharge data, and thus was not utilized for discharge reconstruction.

Table 2-5 Equations used to calculate Drumtee discharge according to the relationships created between the ISCO discharge and the PT stage height data, and the SEPA gauging data at Newmilns. Equations are obtained from Coleman (2017). ST is short for stage height.

Relationship	Equation
ISCO-PT	$Q_{ISCO} = 3.19PT_{ST}^2 - 1.32PT_{ST} + 0.087$ ($p < 0.001$, $R^2 = 0.92$)
ISCO-SEPA	$Q_{ISCO} = 0.075Q_{SEPA} + 0.032$ ($p < 0.001$, $R^2 = 0.89$)

2.3 Laboratory methods

The following section outlines the measurement protocols for water samples after collection from the field. This comprises analysis for DOM, nutrients (SRP and TON), total dissolved Fe and dissolved oxygen (DO).

2.3.1 DOM analysis

2.3.1.1 Preliminary preparation

Prior to any DOC analysis preliminary blank analysis was undertaken to assess the appropriate preparation for samples, including decisions of water sample containers, filter paper rinsing procedure and potential impacts of filter pore size on UV-visible absorbance. The full details are described in Chapter 3 as a part of method developments in this study. The following preparation work was conducted according to the developed protocols.

Particulate material was removed from DOM water samples through the use of GF/F filter paper (Whatman, glass microfiber, 0.7 µm) in a vacuumed system. Commonly-used filters for DOC preparation range between 0.22 – 0.7 µm. In my research group the 0.7 µm GF/F filter has been always used, largely because often radiocarbon analysis of samples are carried out and the GF/F filter is used routinely in the national lab that carries out such analysis. This choice of the filter was maintained in this study to keep the protocol consistent with previous DOC analysis in the group and be able to compare results.

To minimise contamination, filter papers were pre-combusted at 480 °C for 8 hours and rinsed using DI water five times (around 25 ml each time) before filtration (see Chapter 3, Section 3.1.3 for details). The last rinse was measured by UV-visible spectrophotometer at 254 nm to check the absorbance was less than 0.005 cm^{-1} and so small enough to proceed with sample filtration. If the suspended matter load was high, sometimes more than one filter paper was needed. Filtered samples were kept in the cold room (4 °C) in the dark to prevent DOM biodegradation during storage, but were allowed to adjust to room temperature before any measurement was made. These filtered samples were shared for [DOC] measurement, optical analysis (UV-visible and fluorescence analysis) and [Fe] measurement.

2.3.1.2 [DOC] measurement

For measurement of [DOC], it is necessary to remove dissolved inorganic carbon (DIC). This was achieved by titrating the filtered water samples to pH 3.9 by using 0.01 M H_2SO_4 with a Mettler DL20 compact auto-titrator. The exact volume of each titrated sample (normally around 50 ml) and volume of H_2SO_4 used, were recorded for volume correction of sample dilution, which reduced [DOC] as a result of adding acid. Following the titration, samples were degassed in an ultrasonic water bath for 30 minutes to remove existing CO_2 and remaining trace sources of DIC.

[DOC] of prepared water samples was measured using a Thermalox™ TOC Analyser. 30 µl of the degassed water sample was injected into a furnace which was at a high temperature (680 °C) and saturated with oxygen. All DOC in the water sample was oxidised to CO_2 and subsequently detected by a built-in non-dispersive infrared detector. The quantity of CO_2 was converted into [DOC] by using a standard calibration line. Prior to each measurement, standard carbon solutions (normally five for each analysis run) were prepared automatically by the analyser by dilution of a 1000 mg l^{-1} TOC stock solution of potassium hydrogen phthalate to a series of concentrations. The concentrations of standard solutions were determined according to approximate [DOC] estimation of water samples, which generally ranged from 5 – 80 mg l^{-1}. Standard solutions

were included in each run as samples to assess the linearity of instrument. Furthermore an ~ 20 mg l^{-1} check standard prepared from TOC stock solution manually, together with DI water blank were analysed every ten samples throughout the run, which allowed a check of instrument stability and corrections for drift during the analysis to be made if needed (this was rare). Up to three replicated measurements of each sample were made and data was acceptable if the relative standard deviation was smaller than 2%. The limit of detection of the TOC analyser is 1 mg C l^{-1}.

2.3.1.3 UV-visible measurement

For an initial overview of the UV-visible spectra, filtered field water samples were scanned from 200 nm to 800 nm using a Shimadzu UV-2010 PC. Another spectrophotometer, the Hitachi U-1100, was used to measure light absorbance at specific wavelengths (254, 410, 465 and 665 nm), as it was determined to be more stable and accurate than the Shimadzu. For both systems, water samples were placed in a quartz cuvette with 10 mm path length, with another paired cuvette filled with DI water as reference. Cuvettes were rinsed three times using DI water before each measurement. All absorbance data was expressed as per centimetre (cm^{-1}) automatically. DI water was measured as a blank control and subtracted from field sample absorbance.

The UV-visible absorbance at different wavelengths was used to generate the following optical parameters: $SUVA_{254}$, $SUVA_{410}$, E_2/E_4 and E_4/E_6. $SUVA_{254}$ and $SUVA_{410}$ were calculated by dividing the absorbance of a DOM sample at 254 nm or 410 nm respectively by its [DOC], and used to assess DOM aromaticity (Weishaar et al., 2003) and the changes in HS which contain complex aromatic structure (chapter 1). E_2/E_4 was calculated as the UV ratio between absorbance at 254 nm and 410 nm, considered to reflect the intensity of UV-absorbing aromatic rings to HS colour (Zepp and Schlotzhauer, 1981; Selberg et al., 2011; Graham et al., 2012). E_4/E_6 is the ratio between absorbance at 465 nm and 665 nm, and used as considered to identify differences in the proportion of humic and fulvic acids between samples (Thurman, 1985; Spencer et al., 2007; Moody et al., 2013).

2.3.1.4 Fluorescence EEM

Fluorescence measurements of the filtered samples were performed using a Shimadzu RF-5301-PC Spectrofluorophotometer, with samples placed in a 10 mm quartz cuvette with four transparent windows. Spectral correction for the fluorescence spectrometer was carried out using standard solutions (certified by BAM Federal Institute for Materials Research and Testing) prior to measurement. Samples were excited by a light source (Xenon lamp) from 230 nm to 455 nm at 5 nm increments, and the emitted light was

measured at each excitation wavelength from 280 nm to 630 nm at 1 nm increments, to generate 3D EEMs for each individual sample.

The range of emission/excitation wavelength was chosen to eliminate primary and secondary Raman and Rayleigh-Tyndall scatter, which have more scatter than the signal or may be noisy and/or likely to exert disproportionate leverage on the subsequent PARAFAC modelling for data analysis. An EEM of a blank control with DI water was generated on each day of measurement, and subtracted from the EEMs of water samples. Further, Raman scans of blank DI water at 350 nm excitation wavelength, from 370 nm to 428 nm emission wavelengths were collected and the integrated area under the Raman scatter peak was used to normalise blank subtracted EEMs during modelling. Fluorescence intensities were therefore reported in Raman units (RU).

Fluorescence intensity normally increases linearly with [DOC] for very dilute samples, however when there is high [DOC], the increase may not be linear and fluorescence quenching can happen. This occurs when the emitted radiation is re-absorbed by the sample on its way in or/and out of the cuvette, leading to the reduction of the detected amount of excitation/emission light. In order to minimise this so-called 'inner-filter effects (IFE)', water samples with high [DOC] were diluted before fluorescence measurement if the UV absorbance at 254 nm was larger than 1.5 cm^{-1}. The absorbance spectrum was used to calculate a matrix of correction factors for EEMs. The dilution factor was accounted for the final fluorescence intensity in the PARAFAC modelling.

By doing these steps, system stability and bias correction were accommodated, and EEMs were ready to use for further data analysis using the PARAFAC model (see section 2.4.1).

2.3.2 SRP and TON analysis

2.3.2.1 Filtration procedure

Water samples to be measured for the concentrations of SRP ([SRP]) and TON ([TON]) were normally filtered within four hours after sampling. 50 ml of water sample was filtered using a Whatman® nylon membrane filter paper (0.2 µm) in a vacuum system. There is no strict definition of the pore size used for SRP and TON concentrations and the decision was made in this project to follow the same filter size as used by Murray (2012) who also worked in these catchments. To reduce contamination during filtration, filter papers were first rinsed with about 50 ml DI water, followed by 25 ml field water sample to soak and condition the filter paper. Finally 50 ml water sample was filtered and collected in a PETE centrifuge tube. Five filtrates of DI water were collected as blank

controls, so that background contamination could be corrected from the final results. All filtrates were stored in the cold room at 4 °C before analysis which normally took place within next two days.

2.3.2.2 [SRP] and [TON] measurement

SRP and TON concentrations were determined by colorimetric methods using a Technicon Autoanalyser II, where reagents reacted to produce a coloured product whose absorbance was then measured. The concentration was then calculated by reference to the absorbance of a standard solution. Additionally, in order to avoid the background interference of naturally highly-coloured samples (e.g. dark water samples with high [DOC] in this study), which may absorb light at the same wavelength to SRP/TON, background colour correction was required, as described in detail in Murray (2012). The limits of detection for [SRP] and [TON] analysis were 0.15 µg l^{-1} and 0.33 µg l^{-1} respectively (Bazeley, 2013).

Methods for [SRP] analysis

SRP analysis was conducted using the ammonium molybdate-ascorbic acid method (Murphy and Riley, 1962) suitable for the low level analysis of SRP in this project. The ammonium molybdate reacts with orthophosphate ion in acidic solution to form phosphomolybdic acid, which gives a lightly yellow coloured product. This product is then reduced by reacting with ascorbic acid to generate an intensive blue colour. The absorbance of this coloured product was measured at 690 nm light wavelength, and the non-colour corrected [SRP] was calculated referring to the absorbance of standard solutions automatically by the analyser.

Methods for [TON] analysis

Total oxidised nitrogen (TON) includes both nitrate (NO_3^-) and nitrite (NO_2^-) in the sample. Normally there are only trace amounts of nitrites in natural waters therefore [TON] can be interpreted to represent the maximum [NO_3^-].

For TON analysis, a method based on the reaction in Mullin and Riley (1955), and the automated methods of Henriksen (1965) and Best (1976), was used. NO_3^- was reduced to NO_2^- by hydrazine under alkaline conditions and copper (II) is used as the catalyst. Subsequently the total NO_2^- sample was treated with sulphanilamide and N-1-napthylenediamine dihydrochloride under acidic conditions to form a pink colour. The

absorbance of the pink colour was measured and recorded at 530 nm wavelength, and calculated with the reference to the absorbance of standard nitrate-N solutions.

Standard procedure for non-colour corrected concentrations

The standard solution and the series of filtered water samples were run in duplicate, together with five blank control solutions (to obtain potential background contamination from filtration process) in a same run. For SRP analysis, a 0.1 mg l^{-1} standard solution was prepared by the appropriate dilution of a 1000 mg l^{-1} phosphate-P standard stock solution, whilst a standard solution for TON (1 mg l^{-1}) was prepared from the dilution of a 1000 mg l^{-1} nitrate-N stock solution.

Background colour correction

The measurement of nutrient concentration by colorimetry is based on the colour produced at a certain wavelength when reagents react with the analysed samples. However, when analysing naturally highly-coloured water samples, the background colour may interfere at the same wavelength, resulting in an increased absorbance and thus an overestimation of the nutrient concentration. Therefore background colour correction was required, and carried out using the method of Murray (2012) whereby the samples were rerun in the absence of the colour producing reagents. No coloured product was formed and only the background colour detected. The true sample concentration can be quantified by subtracting the background colour signal from the non-colour corrected sample signal. This correction is based on the assumption that the signals produced by the reaction colour and background colour are additive.

2.3.3 Total dissolved Fe concentration

A Perkin-Elmer AAnalyst 100 Atomic Absorption Spectrophotometer (AAS) was used to quantify the total dissolved Fe concentration [Fe] in the stream water samples filtered through 0.7 µm GF/F filter papers (glass microfiber). The total dissolved Fe comprises free Fe^{2+} and Fe^{3+} ions and organic-bound Fe. The absorption wavelength was set to 248.3 nm as Fe atom absorbs light precisely at this wavelength. The absorption is proportional to the concentration. Therefore, Fe standard solutions with concentrations from 1 – 6 mg l^{-1} were prepared using 1000 mg l^{-1} Fe stock solution and applied to create the calibration line for Fe absorption measurement according to the linear relationship between absorbance and concentration. DI water and the 5 mg l^{-1} Fe standard solution were measured every five samples to check the stability of AAS and any potential contamination carried on from previous samples. Three replicate measurements were

made for each sample and the mean value was taken when the standard deviation was smaller than 0.005 mg l^{-1}.

2.3.4 DO concentration

DO concentration [DO] was measured using a dissolved oxygen (DO) meter (HQ10 Hach Portable LDO™) with a luminescent sensor. Collected stream water samples or incubated samples were poured into a 100 ml baker to allow insertion of the DO sensor. As directed by the manufacturer, seven replicate readings were taken for each sample when the meter reading was stable, and the mean values were taken as representative. The observed [DO] change was used to calculate biodegradable DOC loss using the equation: degraded C (mg l^{-1}) = [DO]/32*12 (mg l^{-1}).

2.4 Data processing

2.4.1 PARAFAC fluorescence model

Instead of conventional 'Peak Picking' (Coble et al., 1993), a parallel factor analysis PARAFAC model (Bro 1997; Stedmon et al. 2003; Fellman et al. 2010) in the DOM Fluor Toolbox (version 1.7; Stedmon & Bro 2008) in Matlab was used to examine the complete EEM data and identify discrete independent fluorophores of the EEMs.

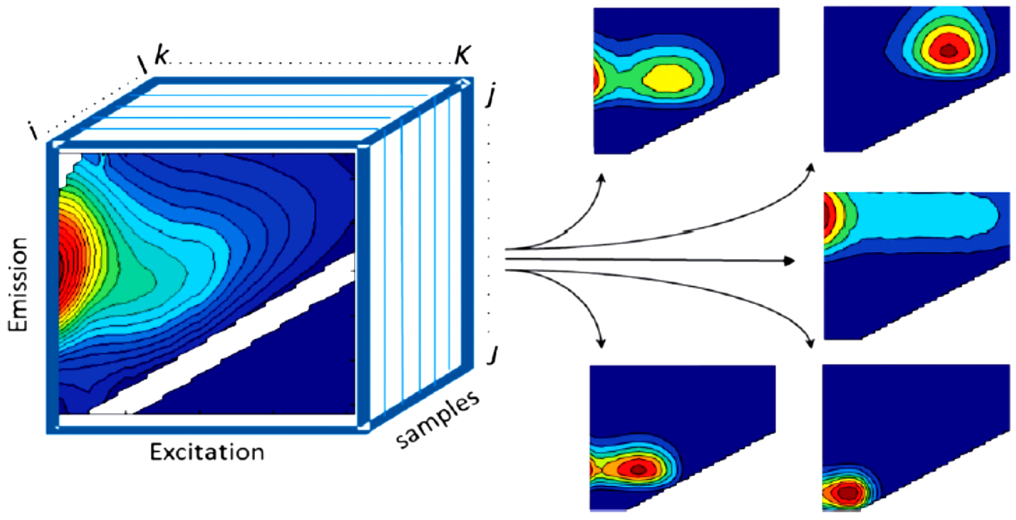


Fig. 2-7. EEM dataset arranged in a threeway structure and decomposed into five PARAFAC components. The i, j and k correspond to the sample, emission and excitation modes (Murphy et al., 2013).

EEMs are typical three-way arrays which consists of sample × excitation wavelength × emission wavelength (Fig. 2-7). PARAFAC analysis is a generalization of principle

component analysis (PCA) to higher order arrays, which statistically decompose EEMs into different independent groups of fluorescent components (Cory and Mcknight, 2005; Stedmon and Markager, 2005a). The following description of PARAFAC algorithm is referred from Bro (1997) and Stedmon et al. (2003). PARAFAC analysis of a three-way dataset decomposes the data signal into a set of trilinear terms and a residual array:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk}$$

Where i = 1, …, I; j = 1, …, J; k = 1, …, K

In the equation, x_{ijk} is the data point corresponding to the ith sample at the jth variable on mode 2 and at the kth variable on mode 3, and e_{ijk} is the residual representing the variability not accounted for by the model. In the case of a fluorescence excitation-emission matrix, the i, j and k correspond to the sample, emission and excitation modes, respectively (Fig. 2-7). x_{ijk} is the intensity of fluorescence for the ith sample at emission wavelength j and excitation wavelength k. a_{if} is directly proportional to the concentration of the fth analyte in the ith sample. b_{jf} is linearly related to the fluorescence quantum efficiency (fraction of absorbed energy emitted as fluorescence) of the fth analyte at emission wavelength j. Likewise, c_{kf} is linearly proportional to the specific absorption coefficient at excitation wavelength k. The residual matrix e_{ijk} represents the variability not explained by the model.

When conducting PARAFAC analysis (using Matlab software), an initial preliminary analysis was performed to identify and remove outliers from the database (Stedmon et al., 2003). A sample would be considered as an outlier if it contained any instrument error, or if it was properly measured but the result behaved rather different from other samples, which could be determined by calculating its loading leverage using the DOM Fluor Toolbox (Murphy et al., 2013). Then a series of PARAFAC models consisting of 3 – 7 components were produced by the DOM Fluor Toolbox based on the remaining samples, and tested for validation by performing half-split analysis (a built-in function in the Fluor Toolbox) to confirm that a PARAFAC model is appropriate to produce identical models from independent subsamples of the dataset (Murphy et al., 2013).

The verified model generated a maximum fluorescence loading (Fmax) for each component, which was normalized in Raman unit (RU), corresponding to their position. For analysis to correct for [DOC] impact on model loadings, the relative distribution of

each component Fmax as the percentage of total fluorescence loading (%Fmax) was also calculated (i.e., [FmaxCi/ΣFmaxC1-6] × 100%).

2.4.2 DOC and Fe fluxes

Scaling discharge to catchment area

All catchments sampled in this study were adjacent in a west-east array such that it was considered that specific discharge would be similar in each sub-catchment. Thus, discharge in the Drumtee sub-catchments (D-WF and D-FF) and other Whitelee catchments (WL14, 15, 16 and 1) can be derived by scaling Drumtee discharge to catchment area, using the below Equation 2-1:

$$\boldsymbol{Discharge = \frac{Area_{catchment}}{5.7\ km^2} \times Discharge_{DT}} \qquad \textbf{Equation 2-1}$$

In the equation, $\boldsymbol{Area_{catchment}}$ is the catchment area corresponding to where discharge is scaling for, while 5.7 km^2 is the Drumtee area. $\boldsymbol{Discharge_{DT}}$ is the measured discharge at Drumtee confluence site. This equation is established upon the assumption that changes between catchment area and discharge is linear, which is supported by the linear relationship observed between Drumtee and SEPA gauging discharge data (Table 2-5). An example is shown here for sub-catchment D-WF (3.9 km^2):

$$\boldsymbol{Discharge_{D-WF} = \frac{3.9\ km^2}{5.7\ km^2} \times Discharge_{DT}}$$

Specific DOC flux

Downstream annual DOC exports from the long-term sites were calculated using 'Method 5' described in Equation 2-2, which utilises flow weighted mean concentrations and mean annual discharge (Dinsmore et al., 2013; Walling and Webb, 1985) and has become a popular and most recommended interpolation method for calculating river load fluxes (Johnes, 2007; Pawson et al., 2012). In the equation, $\boldsymbol{C_i}$ is the instantaneous [DOC] associated with $\boldsymbol{Q_i}$, the instantaneous discharge, $\boldsymbol{Q_r}$ is the annual mean discharge and $\boldsymbol{n}$ is the number of samples analysed.

$$\boldsymbol{Load = Q_r \times \frac{\sum_{i=1}^{n}[C_i \times Q_i]}{\sum_{i=1}^{n} Q_i}} \qquad \textbf{Equation 2-2}$$

Specific annual DOC flux (g m^{-2} $year^{-1}$) was further calculated by dividing annual DOC export by the corresponding catchment area, and estimated for each hydrological year from October 2011 – April 2016 (noting that the 2016 year did not comprise 12 months of observations as the discharge recording was stopped in April 2016).

Specific Fe flux

As [Fe] was only measured for the short-term across all sites (Drumtee sub-catchments: 2014 – 2015; Whitelee long-term sites: 2014- 2016), it was considered more appropriate to compare Fe flux for each month. In this case 'Method 5' would not be suitable for monthly Fe flux estimation, as there was only one [Fe] measurement every month. Therefore, specific monthly Fe fluxes (g m^2 $month^{-1}$) at all sites were estimated by multiplying the instantaneous concentration on sampling days by corresponding specific monthly discharge. Concentration of Fe was assumed to be constant for the time period before the next sample was taken.

3 Method development

This section outlines the method developments carried out in order to have the best UV-visible measurement, and to determine the best approach to quantify DOC biodegradation in the laboratory incubation experiments.

3.1 UV-visible measurement

3.1.1 Sample containers examined for UV-absorbing

UV-absorbing chemical leakage from sample containers is undesirable as contaminates the sample. Therefore before sampling a set of experiments were carried out with some commonly used water containers (polypropylene centrifuge tubes, polypropylene wide mouth 100 ml containers, unused milk bottles and 300 ml PETE drinking water bottles), to assess if they released UV-absorbing substances. These were chosen because they were cheap to buy (many field samples were needed) and some had been used before in the time series generation.

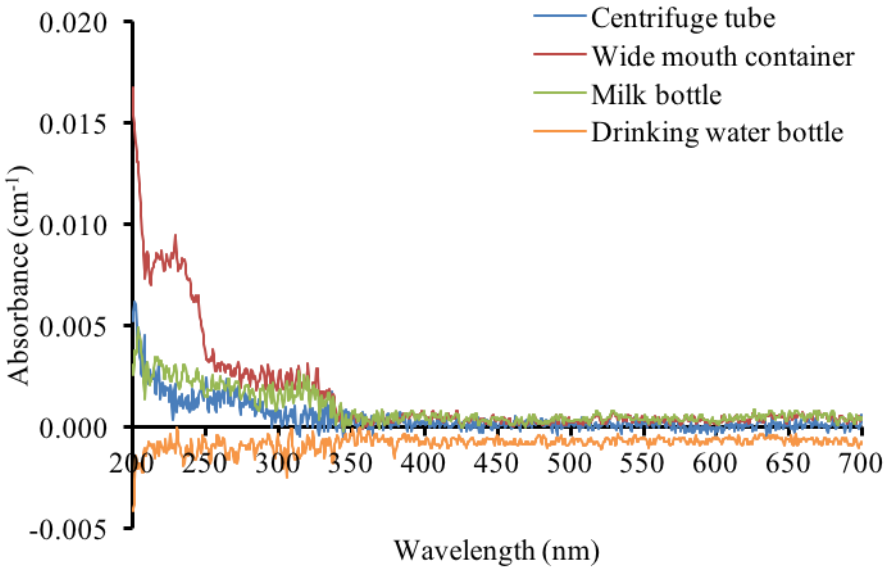


Fig. 3-1. Typical UV-visible spectra (200 – 700 nm) of DI water stored in different containers for 24 hours. Three replicates of stored water in each container were measured and mean values are presented. The negative value is considered to relate to the baseline setting.

All containers were rinsed with tap water and deionized water 3 times respectively and kept for 24 hours filled with deionized water. Absorbance measurement from 200 – 700 nm was conducted on three replicates of stored water from each single container, using a Shimadzu UV-2010 PC spectrophotometer.

The results revealed that all these containers showed negligible absorbance at longer wavelengths from 350 – 700 nm, but leached traces of substances which absorbed UV light mainly at from 200 to 350 nm. The absorbance value of water stored in the wide mouth container was much higher (the max > 0.015 cm^{-1}) at lower wavelengths than the other containers. The spectrum had a shoulder at about 250 nm which would interfere the measurement of absorbance by DOC (e.g. $SUVA_{254}$). The water from the centrifuge tube and milk bottle showed UV absorbance smaller than 0.005 cm^{-1}. The smallest UV absorbance was produced by water from the PETE drinking water bottle (ranging from -0.012 to 0.000 cm^{-1}), indicating it had the least leakage of UV-absorbing materials during the 24-hours storage. The negative value is considered to be related to drift in the instrument baseline. Therefore, the PETE drinking water bottles was chosen as containers for sampling and storage of those water samples which would be measured for UV-visible absorbance.

3.1.2 Filter material and pore size

The effect of the filter material and pore size on UV-visible absorbance was investigated. Filter papers tested included the commonly used Supor membrane filters (Sigma-Aldrich®, 0.2 µm), GF/F filters (Whatman® glass microfiber, 0.7 µm, ashed) and GF/C filters (Whatman® glass microfiber, 1.2 µm, ashed). Water samples were collected from the Drumtee confluence, D-WF1 and D-FF1 sites on November 5th, 2013. Each filter paper was rinsed five times (around 25 ml for each rinsing) with DI water, before being used for filtration. Three replicates of filtrates were made of each filter paper type and measured for UV-visible absorbance at from 200 – 800 nm. Unfiltered water samples were also measured.

Across the sites, the spectra of unfiltered samples were above those of the filtered ones, which would be expected as particles and larger DOM also absorb or reflect UV light. However for each site, the spectra of filtered samples with different filters overlapped each other. For each site, the absorbance difference was small (ranging from 0 to 0.010 cm^{-1}) across the wavelengths of interest (254 nm, 465 nm, 665 nm) between pore size of 1.2 µm (GF/C) and 0.7 µm (GF/F) (Table 3-1). Although samples treated with Supor (0.2 µm) produced less absorbance (except for D-FF1), the difference across wavelengths between GF/F and Supor was negligible (0.001 – 0.03 cm^{-1} for D-WF1 and confluence site; -0.003 - -0.002 cm^{-1} for D-FF1). The small difference might occur because the 0.2 µm filtration screened out bacteria and colloidal particulate materials relatively more thoroughly than the other two (Murray, 2012).

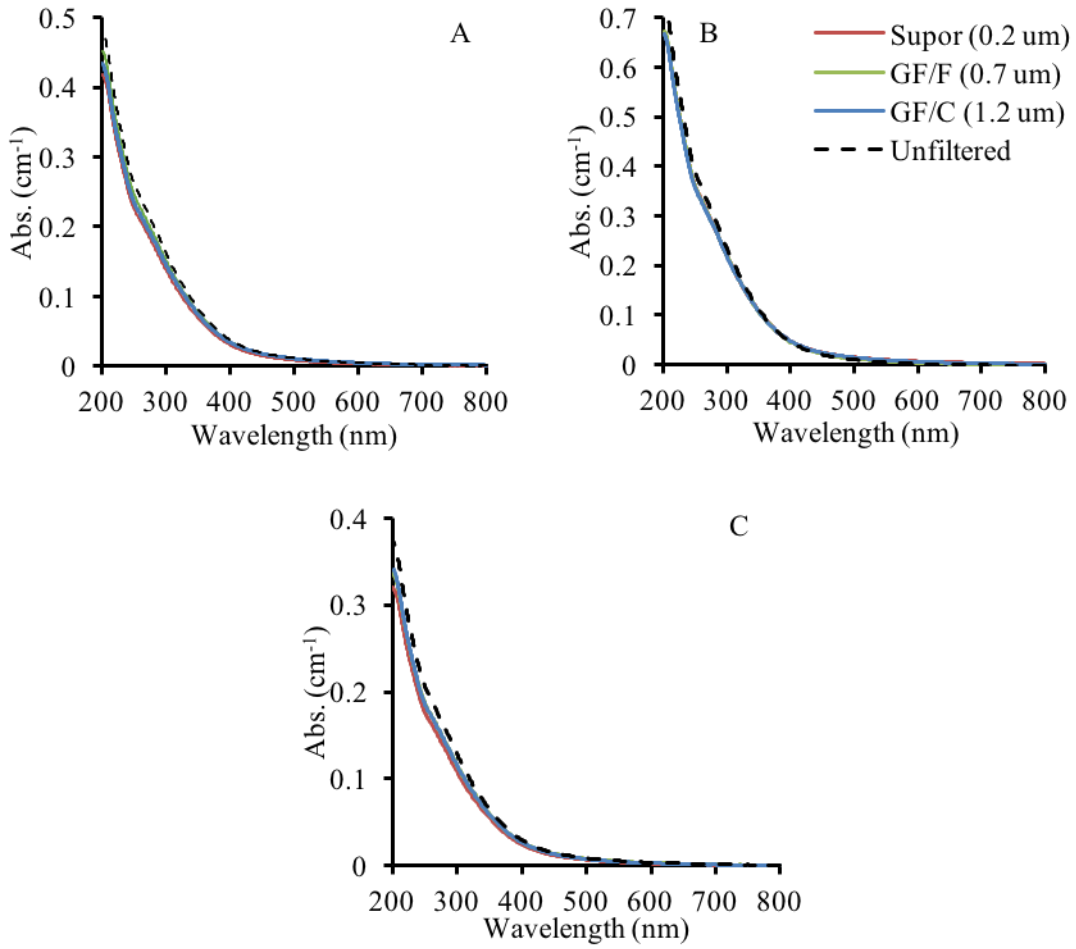


Fig. 3-2. Spectra of water samples collected from (A) confluence, (B) D-FF1 and (C) D-WF1. Mean values of the replicated filtrates (n = 3) were presented. Spectra of filtered samples much overlapped each other.

Table 3-1 UV-visible absorbance of samples from the confluence, D-FF1 and D-WF1 sites in Drumtee. The interested wavelengths (254 nm, 465 nm and 665 nm) are shown for comparison as examples. Average values were calculated for each filtrate (n = 3 replicates of filtrate for each filter paper were generated). The pore sizes for GF/C, GF/F and Supor are 1.2 µm, 0.7 µm and 0.2 µm respectively.

Absorbance (cm^{-1})	Confluence			D-FF1			D-WF1		
	GF/C	GF/F	Supor	GF/C	GF/F	Supor	GF/C	GF/F	Supor
Abs_{254}	0.248	0.238	0.218	0.346	0.348	0.350	0.180	0.181	0.170
Abs_{465}	0.015	0.015	0.012	0.020	0.017	0.020	0.011	0.012	0.010
Abs_{665}	0.002	0.003	0.001	0.003	0.001	0.004	0.002	0.002	0.001

These results suggested there was little impact of filter material or pore size on UV-visible absorbance. Apart from GF/C with 1.2 µm pore size, the other two filters are both commonly used in research for aquatic DOM analysis. However Supor membrane filters

cannot be combusted to get rid of potential contamination. Therefore, the GF/F filter (0.7 μm) was considered a better choice for filtration.

3.1.3 Filter paper rinsing

As UV-visible measurement is very sensitive to potential contamination, a thorough rinsing procedure is required to ensure the analysis quality. Here tests were undertaken to explore the effective rinsing method for the filter paper (GF/F, Whatman® glass microfiber, 0.7 μm, ashed) used for UV-visible measurement. The GF/F filter paper was rinsed with 25 ml deionized water sequentially five times, making 125 ml in total. Each filtrate from rinsing was analyzed with the Shimadzu UV-2010 PC spectrophotometer from 200 – 800 nm.

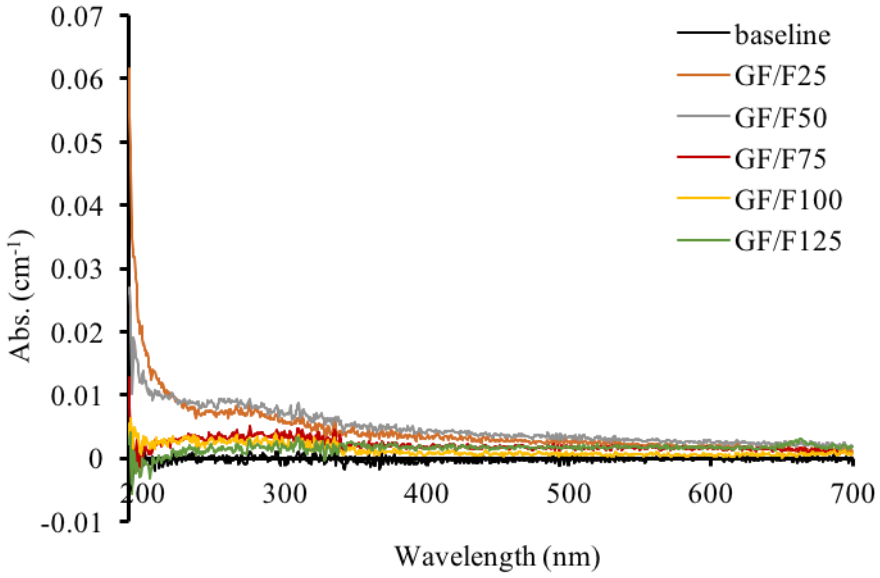


Fig. 3-3. UV-visible absorbance spectra of filtrates from filter rinsing. The filter paper was rinsed with 25 ml deionized water sequentially five times, and 125 ml in total.

All filtrates showed similar spectra trends and rinsing results (Fig. 3-3), which is that the general spectra at short wavelengths (about 200 – 350 nm) had much larger UV absorbance values in the first two rinses, but decreased noticeably with more rinsing. For the last three rinsing with 75 ml, 100 ml and 125 ml respectively, the absorbance spectra of filtrates were very close, and the one from the last rinsing was almost close to the baseline. This supports the idea that filter papers need thorough rinsing before being used for sample filtration. As the spectra showed obvious absorbance mainly at around 200 – 350 nm, and 254 nm is a wavelength that is widely used in research for DOM analysis, the absorbance at 254 nm was chosen to check the rinsing results of filter papers for all UV-visible measurements in this study. It was considered acceptable if the absorbance was smaller than 0.005 cm^{-1}.

3.1.4 Sources of variability of E_2/E_4 and E_4/E_6

Variation of the UV absorbance and calculated parameters can be produced from several experimental processes including field sampling, sample storage, preparation (e.g. filtration) and UV measurement. In the preliminary evaluation of UV parameters, E ratios (E_2/E_4 and E_4/E_6) showed greater intra-stream variation (indicated by larger standard deviation) than the SUVAs. Therefore the total possible variation for UV absorbance and E ratios potentially arising from the whole field and laboratory process was examined.

Methods: Ten replicate water samples were collected from site D-WF1 and D-FF1 during one hour at five-minute intervals on November 18th, 2014. Each sample was collected randomly from depth within 10 cm to the water surface. Water samples were filtered with rinsed GF/F filter paper (0.7 μm, ashed) immediately after being returned to the lab and filtrates were stored in the cold room at 4°C. Samples were warmed up to 25°C using the incubator before measurement to reduce the impact caused by temperature differences. Sample absorbance was measured using Hitachi UV spectrophotometer at specific wavelengths (254 nm, 410 nm, 465 nm and 665 nm), and hereafter referred to Abs_{254}, Abs_{410}, Abs_{465} and Abs_{665}. Samples with absorbance at 254 nm higher than 2.0 cm^{-1} were diluted 1:1 with DI water.

Standard deviation (SD) and relative standard deviation (RSD%) were calculated to quantify the amount of variation for absorbance at each wavelength and measurement repeatability. For E_2/E_4 and E_4/E_6, RSD% and maximum probable error (MPE) were used to analyze the repeatability and combined variation. SD was calculated using the following formula:

$$\boldsymbol{SD} = \sqrt{\frac{\mathbf{1}}{\boldsymbol{N}-\mathbf{1}}\sum_{\boldsymbol{i}=\mathbf{1}}^{\boldsymbol{N}}(\boldsymbol{x_i}-\bar{\boldsymbol{x}})^{\mathbf{2}}}$$

where $\boldsymbol{N}$ is the number of measurement, $\boldsymbol{x_i}$ stands for the individual measurements made, $\bar{\boldsymbol{x}}$ is the average of $\boldsymbol{x}$. RSD% was calculated by dividing the SD by the mean value.

MPE of ratios E_2/E_4 and E_4/E_6 were calculated using the following formula:

$$\frac{\mathbf{\Delta Z}}{\mathbf{Z}} = \sqrt{\left(\frac{\boldsymbol{\Delta X}}{\mathbf{X}}\right)^{\mathbf{2}} + \left(\frac{\boldsymbol{\Delta Y}}{\mathbf{Y}}\right)^{\mathbf{2}}}$$

Δ stands for the SD of $\boldsymbol{X}$, $\boldsymbol{Y}$, $\boldsymbol{Z}$.

In this way variations of each absorbance as well as the parameters used for data analysis caused by sampling, storage, filtration, dilution and measurement were all included and considered in the analysis.

Results: For D-WF1 samples, SDs of absorbance for these interested wavelengths were less than 0.001 cm^{-1} except for Abs_{254} (0.0014 cm^{-1}) (Table 3-2). However at 254 nm, absorbance difference between sites were normally ≥ 0.01 cm^{-1}. The small SDs indicated D-WF1 absorbance had very small absolute variations. All RSD% of D-WF1 absorbance was small (≤ 0.5 %) apart from that for Abs_{665} (2.9 %). The greater Abs_{665} RSD% (but still smaller than 5.0 % and thus acceptable) was because this absorbance was always so small that little difference can make the SD relatively larger to the mean Abs_{665}. Small SDs and RSD% for different wavelengths suggest a good repeatability of D-WF1 samples at these interested wavelengths.

Table 3-2 Mean absorbance, SD and RSD% at wavelengths 665 nm, 465 nm, 410 nm and 254 nm of the D-WF1 and D-FF1 sample replicates (n = 10) respectively.

	D-WF1			D-FF1		
Absorbance	Mean (cm^{-1})	SD (cm^{-1})	RSD (%)	Mean (cm^{-1})	SD (cm^{-1})	RSD (%)
Abs_{665}	0.011	0.0003	2.9	0.022	0.0005	2.4
Abs_{465}	0.083	0.0003	0.4	0.145	0.0006	0.4
Abs_{410}	0.173	0.0005	0.3	0.296	0.0010	0.3
Abs_{254}	1.291	0.0014	0.1	2.326	0.0060	0.3

The SDs of D-FF1 absorbance at different wavelengths ranged from 0.0005 to 0.0060 cm^{-1} (Table 3-2). Only Abs_{254} SD was greater than 0.001 cm^{-1} but was smaller than the commonly observed Abs_{254} difference between sites. Similarly the absorbance of D-FF1 replicates at most wavelengths showed negligible RSD% (0.3 – 0.4 %). Although a greater RSD% (2.4 %) was shown for Abs_{665}, it was still small enough (< 5%) not to interfere the analysis of real absorbance difference between sites.

Table 3-3 Mean values, MPEs and RSD% of E_2/E_4 and E_4/E_6 for the D-WF1 and D-FF1 sample replicates (n = 10) respectively.

	D-WF1			D-FF1		
Ratio	Mean	MPE	RSD (%)	Mean	MPE	RSD
E_2/E_4	7.4	0.02	0.3	7.8	0.03	0.3
E_4/E_6	7.6	0.22	3.2	6.7	0.16	2.2

MPEs and RSD% of sample ratios E_2/E_4 and E_4/E_6 from both sites are shown in Table 3-3. MPE of E_2/E_4 was 0.02 for D-WF1 and 0.03 for D-FF1. The observed E_2/E_4 differences between sites were usually ≥ 0.1. Additionally a small RSD% existed for both sites (0.3 % for D-WF1, 0.3 % for D-FF1). These results indicate there was a good repeatability in the measurement, and the estimated error would not have much interference on intra-stream E_2/E_4 interpretation.

However, there were larger MPEs for E_4/E_6 in both sites (D-WF1: 0.22, D-FF1: 0.16). Thus differences in E_4/E_6 of < 0.5 could be considered as experimental variability. The RSD% in D-WF1 and D-FF1 was relatively large (3.2 % and 2.2 % respectively) but still suggests a good repeatability of E_4/E_6 measurement at each site.

3.1.5 Interference of inorganic species on UV-visible absorbance

The presence of Fe has long been suggested as a potential interference in measuring UV-visible absorbance of DOM (Doane and Horwáth, 2010), with the characteristic of absorbing light from 200 – 400 nm (Stefánsson, 2007). Apart from Fe, inorganic species such as N and Mn can also absorb light (Weishaar et al., 2003; Xiao et al., 2013). As UV absorbance and relevant UV parameters were used as important approaches in this research, the potential interference of Fe, N and Mn on UV-visible light absorption was tested for correction. The following correction method was adapted from that used in Weishaar et al. (2003).

Methods: To assess whether the presence of Fe^{2+}, Fe^{3+}, NO_2^-, NO_3^- and Mn^{2+} interfere with UV-visible absorbance of DOC, responses in the 200 – 800 nm UV-visible spectra wavelength were measured in pure solutions (5 mg l^{-1}) using a Shimadzu UV-2010 PC. Fe^{2+}, Fe^{3+}, NO_2^-, NO_3^- and Mn^{2+} solutions were made of $Fe(NH4)_2(SO_4)_2•6H_2O$, $NH_4Fe(SO_4)_2•12H_2O$, $NaNO_2$, $NaNO_3$, $MnSO_4•4H_2O$ with blank DI water respectively. In order to not introduce extra interference, solutions were measured within five minutes before any precipitation occurred.

A series of Fe^{3+} addition experiments were further conducted to investigate the detailed impact of Fe^{3+} on UV-visible absorbance. The inorganic salt $NH_4Fe(SO4)_2·12H_2O$ was dissolved in DI water to prepare 100 mg l^{-1} Fe^{3+} solution. Filtered water samples (40 ml) from Drumtee sub-catchments (D-WF and D-FF), and the five long-term sites (WL13, 14, 15, 16 and 1) were mixed with sufficient sample from the 100 mg l^{-1} Fe^{3+} solution and 5 ml 0.01M HCl to make up 50 ml solutions with a background 0.001 M HCl and additional 0 mg l^{-1}, 1 mg l^{-1}, 2 mg l^{-1}, 3 mg l^{-1}, 4 mg l^{-1} and 5 mg l^{-1} Fe^{3+} amendment. HCl was used to prevent Fe^{3+} precipitation potentially caused by the interactions between Fe^{3+} and field

water samples, which was observed in preliminary trials. Although HCl produces a major peak below 210 nm, it is not the wavelength of interest in this study thus it is fine to use. DI water with the same Fe^{3+} (0 – 5 mg l^{-1}) and HCl (0.001M) amendment was made and regarded as a blank. Two replicates were made for each Fe^{3+} solution. Samples were measured for Fe^{3+} concentration using a Perkin-Elmer AAnalyst 100 Atomic Absorption Spectrophotometer, and for UV absorbance at 254 nm and 410 nm using a 1 cm quartz cuvette in a Hitachi U-1100 spectrophotometer, both as described earlier in Chapter 2.

Results: The UV-visible spectra of each inorganic solution showed that, among the analysed inorganic species, negligible absorbance was produced by both Fe^{2+} and Mn^{2+} across the wavelength range of interest (200 – 800 nm) (Fig. 3-4). NO_2^- and NO_3^- absorbed UV from 200 nm to about 250 nm and produced a peak at 210 nm (Fig. 3-4). Since no absorbance occurred at the wavelengths of interest (including 254 nm, 410 nm, 465 nm and 665 nm), the absorbance produced by NO_2^- and NO_3^- was not of concern in this study.

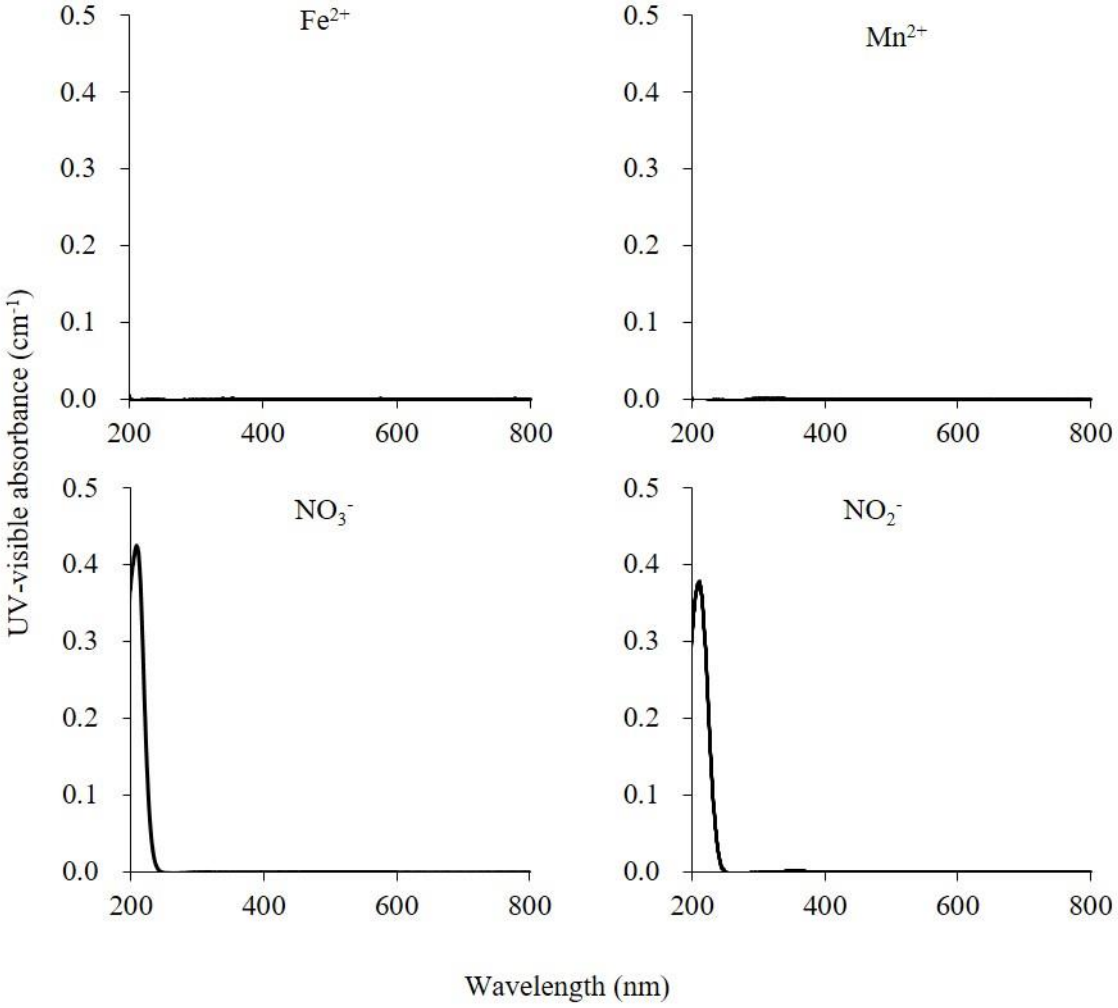


Fig. 3-4. The UV-visible spectra of solutions with Fe^{2+}, Mn^{2+}, NO_3^- and NO_2^- respectively from 200 – 800 nm. The absorbance for NO_3^- and NO_2^- was much higher than other two species, so for equivalent scaling of the four graphs, 1 mg l^{-1} NO_3^- and NO_2^- solutions were used while 5 mg l^{-1} for others.

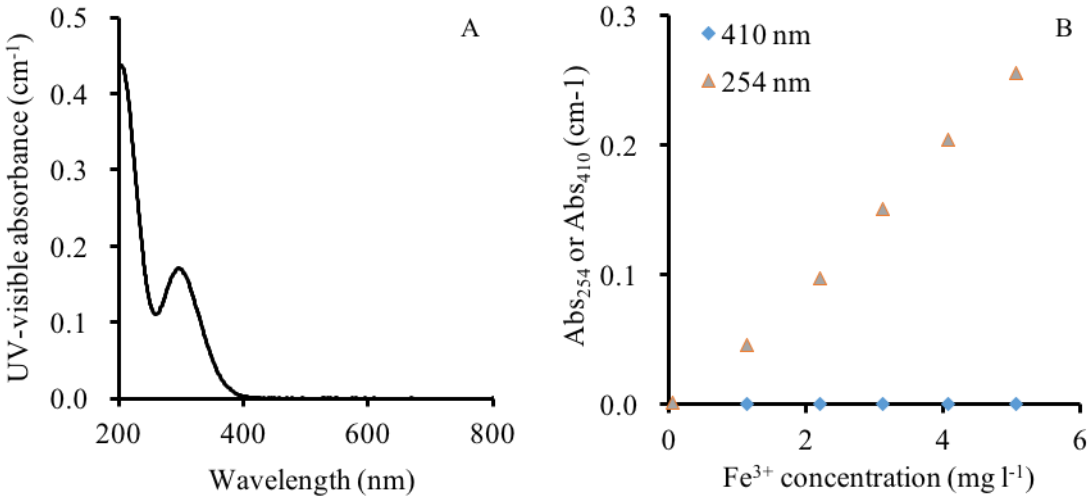


Fig. 3-5. The UV-visible spectrum of Fe^{3+} solution (5 mg l^{-1}) from 200 – 800 nm (A). Impacts of different Fe^{3+} concentrations on UV-visible absorbance at 254 and 410 nm of blank control waters (B).

However, absorbance by Fe^{3+} increased from around 400 nm towards shorter wavelengths and showed peaks at 295 and 205 nm respectively (Fig. 3-5A). Therefore, it was necessary to examine the interference of Fe^{3+} on absorbance at 254 nm and 410 nm, which were wavelengths used in this project in the range potentially impacted by Fe^{3+}. Fig. 3-5B illustrates the absorbance of Fe^{3+} solution at 254 nm and 410 nm, with higher absorbance at shorter wavelength for each concentration analysed (0 – 5 mg l^{-1}). The absorbance at 254 nm ranged from 0 – 0.26 cm^{-1} with [Fe^{3+}] increasing from 0 – 5 mg l^{-1}. The impact was much reduced at 410 nm which is the lower end of visible range, with negligible absorbance (0 – 0.002 cm^{-1}) in all DI water with [Fe^{3+}] up to 5 mg l^{-1}. Therefore, the correction of Fe^{3+}-induced impact on UV absorbance for field water samples was only needed at 254 nm.

The positive linear effect of Fe^{3+} concentration on Abs_{254} evident in the control samples (Fig. 3-5B) was also observed in the amended field water solutions from both Drumtee sub-catchments and long-term monitoring sites (Fig. 3-6). The absorbance difference between streams reflected different starting [DOC], but the slopes of these relationships were very similar across all sites. The coefficient of each [Fe^{3+}]-Abs_{254} equation was then used to correct Abs_{254} for each site respectively, using the measured sample [Fe^{3+}] to guide the correction. Equations listed in Table 3-4 were used for corrections of Fe^{3+} interference on Abs_{254} across all sites.

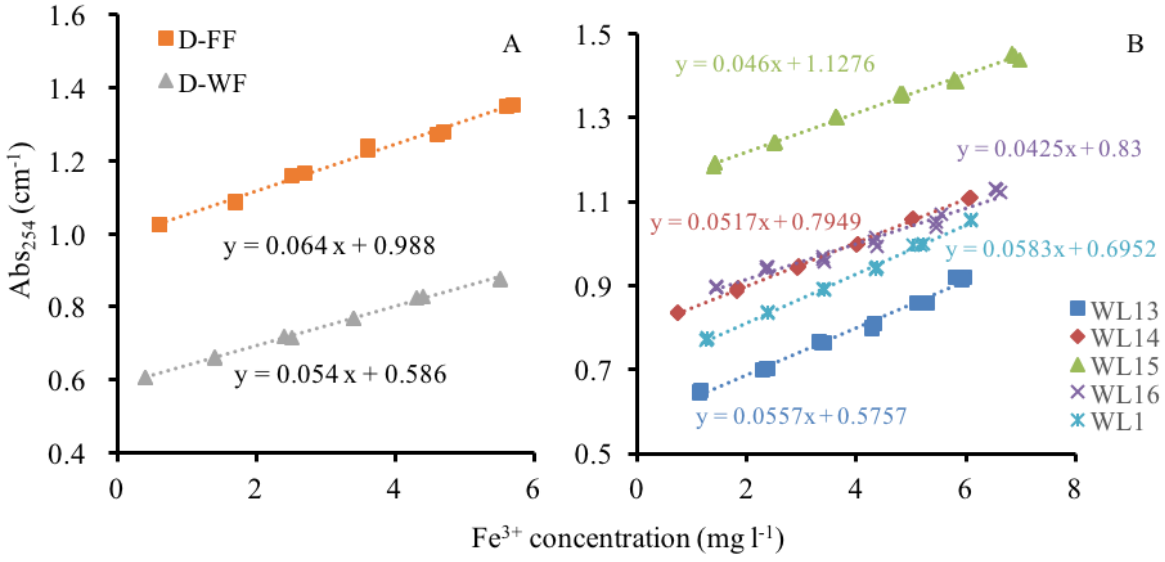


Fig. 3-6. The effect of increasing [Fe^{3+}] on UV absorbance at 254 nm for amended water samples from Drumtee sub-catchments (A) and long-term monitoring sites (B).

Table 3-4 Equations used for corrections of Fe^{3+} interference on UV absorbance at 254 nm. Coefficients slightly varied among different sites.

Site	Correction equation
D-WF	Corrected Abs_{254} = Measured Abs_{254} – 0.054 * $[Fe^{3+}]$
D-FF	Corrected Abs_{254} = Measured Abs_{254} – 0.064 * $[Fe^{3+}]$
WL13	Corrected Abs_{254} = Measured Abs_{254} – 0.056 * $[Fe^{3+}]$
WL14	Corrected Abs_{254} = Measured Abs_{254} – 0.052 * $[Fe^{3+}]$
WL15	Corrected Abs_{254} = Measured Abs_{254} – 0.046 * $[Fe^{3+}]$
WL16	Corrected Abs_{254} = Measured Abs_{254} – 0.043 * $[Fe^{3+}]$
WL1	Corrected Abs_{254} = Measured Abs_{254} – 0.058 * $[Fe^{3+}]$

3.2 DOC biodegradation during laboratory incubation

3.2.1 Introduction

It is essential and fundamental to achieve a reliable and accurate method to assess DOC breakdown during biodegradation incubation, based on which further analysis can be combined to explore the relationships between DOC composition and its biodegradability. There are no standard approaches to quantify the biodegradable DOC (BDOC) concentration in soils yet (Marschner & Kalbitz, 2003) and neither are there in aquatic system to the best of my knowledge. Various parameters have been used as promising proxies for BDOC such as nutrient concentration, amino acid concentration, optical properties and molecular characteristics (Balcarczyk et al. 2009; Wickland et al. 2012; Abbott et al. 2014; Shen et al. 2015; Vonk et al. 2015). For example amino acids are bioactive components of DOM and have been used as molecular indicators of bioavailable DOM in groundwater (Chapelle et al., 2009) and marine environments (Davis et al., 2009).

However more typically, BDOC has been assessed through laboratory bioassay incubation experiments (Vonk et al., 2015). Commonly-used methods in incubation to quantify microbial DOC degradation include DOC consumption (the most widely used method), CO_2 production and dissolved O_2 consumption (McDowell et al., 2006). The design of incubation experiments in different fields are quite diverse including variation in incubation duration, nutrient additions, inoculum, light exposure, temperature and the measure of biodegradation (Table 3-5), and these may affect BDOC quantification. For example the source of inoculum was found not to affect measured BDOC rate (Yano et al., 1998), but biodegradation can be greater when using an inoculum with attached rather than suspended microbes (Trulleyova and Rulik, 2004). From the literature review of previous research, it is found to be more often that incubation is carried out in dark at

15 – 20 °C, with no additional nutrient, and no aeration, but with an inoculum. The water sample is filtered before incubation (mostly with 0.2 or 0.45 um pore size filter). However it is still variable between different researchers, as are incubation duration and the approach to quantify BDOC loss. Thus a suitable incubation approach and conditions needed to be determined before conducting the DOC biodegradation incubation in this research.

Table 3-5 Summary of methods used in BDOC incubation with samples from various environment. As this project is aiming at biodegradation process of DOC in stream waters, more examples are considered that are from aquatic systems.

Samples	Measured	Light & temperature	Additives	Filter (μm)	Aeration	Sampling frequency	Duration (day)	Reference
Subarctic river water	CO_2	15 °C	No nutrient, inoculated	0.45	No	Every 7 days	28	Wickland et al. 2012
Soil leachates	CO_2	Dark, 20 °C	No nutrient, inoculated	0.2	Yes	Firstly 3-day intervals then 14-day	90	Kalbitz et al. 2003
Soil water (bog, forested, wetland and fen)	[DOC]	Dark, 25 °C	Inoculated	0.2	Yes	Before and after incubation	30	Fellman et al. 2008
Reconstituted moorland headwater DOM	[DOC]	Dark, 15 °C	Varied nutrients/inoculums	0.7	-	On 0, 2, 5, 13, 22 and 41 days	41	Stutter et al. 2013
Boreal lake water	[DOC], CO_2 and [DIC]	Dark, 17.5 °C	No nutrient, inoculated	0.45	No	On 1, 3, 6 and 11 days	11	Olefeldt et al. 2013
Boreal peatland well waters	[DOC], CO_2 and [DIC]	Dark, 17.5 °C	No nutrient, inoculated	0.45	No	On 1, 3, 6 and 11 days	11	Olefeldt et al. 2013
Forested coastal headwater stream water	[DOC]	Dark, 20 °C	No nutrient, inoculated	0.2	Yes	Before and after incubation	28	Hosen et al. 2014

3.2.2 Methods development

The main aims of this method development were to determine 1) the most suitable and reliable BDOC measure, 2) the best incubation length for the samples in this project and 3) the general level of DOC biodegradability in the studied stream water. The following specific objectives were also to be achieved: whether to add in additional nutrients and inoculums, and if to use aerated systems (this would be decided according to the BDOC approach used, e.g. aeration would be required for CO_2 production and DO consumption measurements). Temperature was controlled at 20 °C as similar to that in other research. Unfiltered water samples were incubated as to better mimic the original water ecosystem, and to include production from and absorption by particle organic carbon (POC). Overall, there were three phases in this method development, and the following sections will present each phase and relevant results and discussion in a linear manner.

3.2.2.1 Phase 1

Objectives: At Phase 1, two commonly-used BDOC approaches ([DOC] reduction and CO_2 production) were tested for the same incubated samples. This allowed the comparison of the estimated values for decomposed DOC, to determine if the approaches were sensitive and precise enough to quantify BDOC loss. As CO_2 production was measured, a foil-sealed system was used with no aeration in order to avoid potential interference from outside environment. O_2 may be depleted in sealed flasks, and thus a three-week incubation length (which was similar to other incubation research) was tried out to test if O_2 depletion occurred in the time-frame. Different additive conditions for nutrients and inoculum were applied to explore if these options were necessary for my peaty catchment water samples.

Methods: A total of 400 g river bed sediment and soil from different sites (Stream D-WF1, D-WF7, D-FF1 and D-FF5) were collected on May 25th, 2015 and well mixed together to represent a microbial inoculum from the sources that may be found in the river. After removal of large material such as rock fragments, 100 g mixed sediment was re-suspended in 300 ml river water in triplicate, and incubated in open flasks for 3 days at 20 °C with stirring. After 3 days, the replicates were mixed again in a 2 l flask and then left undisturbed for five minutes. This allowed sediments and silt to settle down to the flask bottom, but left the clay suspended in the solution. This solution was used as an inoculum and added to DOC water samples in the required volume when needed.

Sixteen litres of bulk stream water were collected from confluence site using a 16 litre carboy on May 25th, 2015. On return to the lab 110 ml of bulk water was filtered (0.7 µm

ushed Whatman GF/F) immediately for initial [DOC] measurement. Twelve litres of bulk water was divided into four groups (A, B, C and D) with three replicates of each (namely 1 l river water per set) and added to twelve 2 l foil-sealed incubation flasks A1-A3, B1-B3, C1-C3 and D1-D3 (Fig. 3-7). Four different treatments were applied to the DOM solution groups to explore if additional nutrients would be needed: (A) no inoculum added; (B) adding 2.5 ml microbial inoculum; (C) adding 7.3 ml nutrient stock (4.5 ml 100 mg N l-1 $NaNO_3$, 2.8 ml 100 mg P l-1 KH_2PO_4 to provide 4.5 mg l^{-1} N and 0.28 mg l^{-1} P, calculation according to Redfield ratio); (D) adding both 2.5 ml microbial inoculum and 7.3 ml nutrient stock. Another group of three 2 l sealed flasks with 1 l DI water only was used as blank control for incubation. All incubations were carried out in a dark room (to prevent photosynthesis) at 20 °C (stably maintained by a programmed thermometer) for three weeks. A temperature logger was used to monitor the average room temperature profile. Stirring was carried out continuously during the incubation using a magnetic stirrer to facilitate O_2 dissolution in incubated sample waters.

Fig. 3-7. A 2 litre foil-sealed flask used for the incubation. A small glass jar was hung in the headspace to capture CO_2 produced. Totally 15 flasks were used: three replicates in each treatment and one blank control.

For CO_2 detection, a small jar with NaOH solution (10 ml, 1 M) was hung in the headspace of each flask to capture CO_2 produced during incubation (Fig. 3-7). At either 3- or 4-day intervals, 110 ml aliquot of sub-sample was collected from each flask for [DOC] measurement using Thermalox™ TOC analyser. The Jars of NaOH were removed and rinsed with DI water throughout into titration cups quickly, which was then titrated by 1 M HCl to calculate CO_2 production during incubation. The values of CO_2 mass were

converted to the decomposed DOC according to the equation below, where $\boldsymbol{m}$ is the mass of CO_2 or C, and $\boldsymbol{M}$ is the molar mass:

$$\boldsymbol{m_C = \frac{m_{CO_2}}{M_{CO_2}} \times M_C}$$

Three 10 ml 1 M NaOH aliquots were used as blanks for titration, and the mean value was subtracted from the values for incubated samples. The sampling interval was designed as 3 or 4 days to avoid practical work at weekends for health and safety reasons.

Results: Fig. 3-8 shows the results for DOC degradation in the different treatment groups estimated from detecting CO_2 production. Biodegradation was fastest in the first two weeks with similar rate across the groups, and slower thereafter. This may suggest the labile BDOC was decomposed quickly in the first two weeks with little degradation occurred in the last week. During this 3-week incubation 5.62 – 9.24 mg C (20.4 – 33.6 % of total [DOC]) was decomposed in total. Group A, with no additional treatment, had the least amount of biodegraded C (5.62 ± 3.29 mg), while Group B, which had an inoculum, had the most (9.24 ± 1.52 mg). Group C with addition of nutrients degraded more C degradation than the Group D which had both inoculum and nutrients, and group with no treatment (Group A). Larger standard deviations for treatment replicates were observed in Group A in the last two weeks.

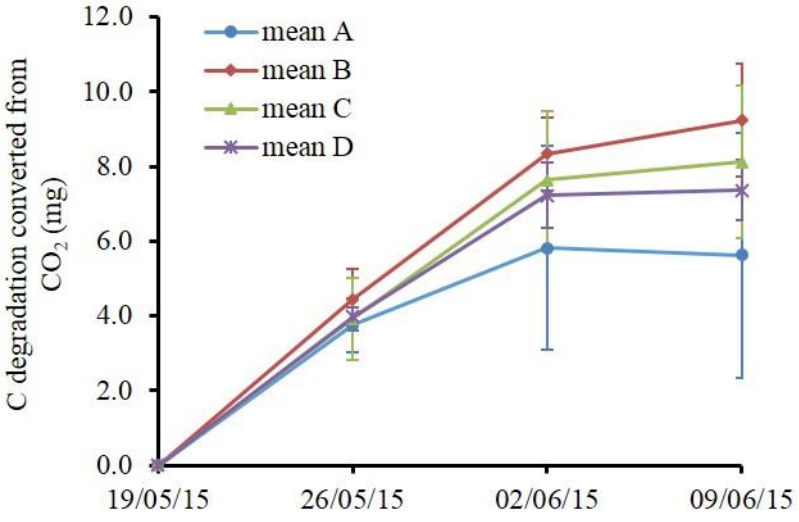


Fig. 3-8. Weekly BDOC loss in each treatment converted from CO_2 production. Mean values of treatment replicates are presented with error bar representing SD. A: no inoculum added; B: microbial inoculum added; C: nutrient stock added; D: microbial inoculum and nutrient stock added.

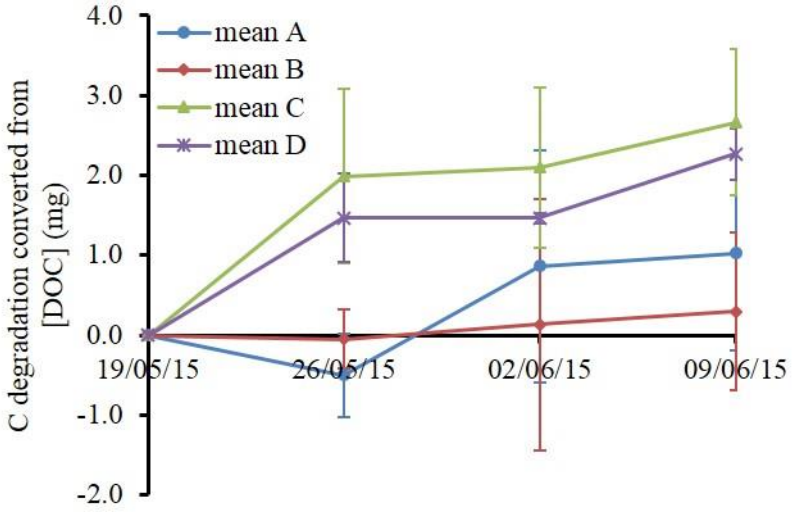


Fig. 3-9. Weekly BDOC loss in each treatment converted from [DOC] changes. Mean values of treatment replicates are presented with error bar representing SD. A: no inoculum added; B: microbial inoculum added; C: nutrient stock added; D: microbial inoculum and nutrient stock added. The initial [DOC] was 27.5 mg l^{-1}.

However different results for DOC biodegradation were generated for the same incubation from the measurement of change in [DOC]. In total there was much less C degraded after three-week incubation, ranging from 0.30 to 2.26 mg (Fig. 3-9). The largest amount of C degradation occurred in Group C with nutrients added, and the smallest in Group B with inoculum added (Group B appeared to have the most C degradation by the CO_2 detection). Different and more variable decomposition trends across groups were indicated from changes in [DOC]. In Groups C and D, the trends were more consistent, with the fastest degradation rate in the first week, and decreasing afterwards. In Group A, a negative value of C biodegradation was shown after the first week incubation.

Discussion: For the measurements of CO_2 production, a relatively large proportion of DOC was degraded during the 21-day incubation. This fast decomposition rate could be too optimistic, as DOC from peaty catchment water is normally more humic and persistent than that in general fresh water (more information shown in Chapter 4). For example there was a small percentage of biodegraded DOC for headwater draining forest and peat (1.63 – 9.30 %) during 20-day incubation (Fasching et al., 2014). It could be possible that dissolved inorganic carbon (DIC) in the forms of HCO_3^- and CO_3^{2-} and free CO_2 also contributed to the relatively large amount of CO_2 captured in the incubation. This amount of DIC needed to be quantified and corrected before continue using CO_2 detection for BDOC measurement as it was a potential interference. This exploration was undertaken at Phase 2.

For the [DOC] measurements, small weekly changes (-0.48 - 1.98 mg l^{-1}) in [DOC] were observed, and within the TOC analyser variation limits (2 mg l^{-1}). The large SD of each [DOC] measurement across four treatments may further support that it was out of the ability of TOC analyser to detect the small changes accurately in the incubation. As a result, [DOC] measurement was not sensitive enough to detect this small amount of C biodegradation, and thus cannot be applied in the incubation experiment for the Drumtee water samples.

Biodegradation of 27.5 mg l^{-1} DOC in 1 l solution theoretically required 73.3 mg O_2, which was much less than the O_2 presented in 1 l headspace (calculations according to ideal gas law at 20 °C). Thus, O_2 limitation may not happen in this incubation system during the 21 days. Further, it was observed that the incubation length of 21-day was long enough to capture the fast and slow DOC decomposition periods. In addition, the literature review shows much biodegradation incubation last for 20-40 days. Therefore, at this stage it was suggested to carry out incubation experiments for 21 days in this research.

Further, no consistent trends in DOC biodegradation changes were observed across different treatments between the two sets of results. However it was observed that addition of nutrients and/or inoculum did not necessarily contribute to more DOC biodegradation. One possible reason for the less clear impacts from nutrient stock could be that whilst microbes consumed nutrients for DOC degradation, some nutrient materials were released from the decomposed DOC at the same time. Thus in order to simplify the experiment design, in the following experiments at Phase 2 and 3, and in Chapter 6, raw water samples were incubated with no nutrients or inoculum addition.

3.2.2.2 Phase 2

Objectives: When measuring CO_2 production to estimate BDOC loss at Phase 1, concerns arose as what source of carbon (DOC or DIC) contributed to the NaOH-captured CO_2. Thus, at this phase, experiments were carried out to analyse if the CO_2 captured during the incubation was partially produced by different forms of DIC (HCO_3^- and CO_3^{2-} and free CO_2) existing in the water samples, and if so to explore the effective correction for this DIC interference.

Methods: The DIC interference was taken into account when using CO_2 measurement for BDOC concentration in previous research (Wickland et al., 2012), by measuring headspace CO_2 concentration immediately after acidifying samples before and after incubation. However, CO_2 in the headspace cannot be directly related to CO_2 in the liquid phase in

the stream, as CO_2 outgassing from water causes the equilibria of the carbonate fractions (CO_2, HCO_3^- and CO_3^{2-}) to shift (Fasching et al., 2014).

Here, DIC interference can be minimized by quantifying the total DIC loss in water samples during incubation (Fig. 3-10). Generally, concentration of different DIC forms (CO_2, HCO_3^- and CO_3^{2-}) can be calculated from the sample pH and total alkalinity according to the equations below:

a. Bicarbonate alkalinity:

HCO_3^- as mg $CaCO_3$ l^{-1} = $(T - 5.0 \times 10^{(pH-10)}) / (1 + 0.94 \times 10^{(pH-10)})$

Where: T=total alkalinity, mg $CaCO_3$ l^{-1}.

b. Carbonate alkalinity:

CO_3^{2-} as mg $CaCO_3$ l^{-1} = $0.94 \times B \times 10^{(pH-10)}$

Where: B = bicarbonate alkalinity, from a.

c. Free carbon dioxide:

mg CO_2 l^{-1} = $2.0 \times B \times 10^{(6-pH)}$

Where: B = bicarbonate alkalinity, from a.

d. Potential total carbon dioxide:

mg total CO_2 l^{-1} = $0.44 \times (2A + B) + C$

Where: A = bicarbonate alkalinity from a, and

B = carbonate alkalinity from b

C = mg free CO_2 l^{-1}.

Alkalinity measurement is a measure of the HCO_3^- and CO_3^{2-} in a water sample obtained by titrating the sample to pH 4.5 with standard acid (0.02 M HCl) and is expressed as mg $CaCO_3$ per liter. 1 ml of 0.02 M acid is equivalent to 1 mg $CaCO_3$. If there is DIC loss in the water samples during incubation, it would suggest the captured C may be partly or even largely derived from the DIC source. An approach would be needed to identify the true amount of CO_2 released from DOC respiration during the incubation.

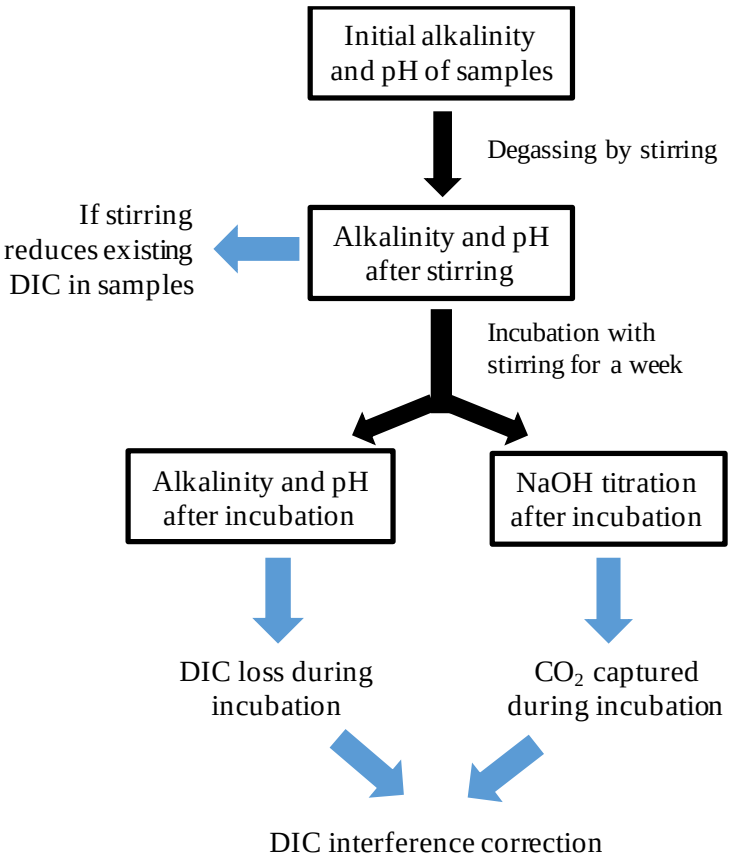


Fig. 3-10. Procedures examine and correct the potential DIC interference to measured CO_2 production during the incubation.

Bulk stream water from the confluence site was collected on August 5th, 2015. Six 50 ml screw-top wide mouth tubs were fully filled with sub-samples from the bulk water without headspace for initial alkalinity measurement. The rest of bulk sample was dispensed into six 2l flasks with equal volumes (1 l) and stirred for three hours with flasks open to expel existing free CO_2. Alkalinity was measured again for six replicates of subsamples of the stirred water just before incubation. This was undertaken to examine if stirring could effectively reduce the existing DIC (including HCO_3^-, CO_3^{2-} and dissolved CO_2).

Afterwards the rest stirred samples were incubated in the sealed flaks (2 l) in dark at 20°C for one week. As the incubation length had already been determined at Phase 1, here this one-week incubation was used only to examine if there was DIC interference. A plastic titration cup with 10 ml 1 M NaOH was hung in each flask to capture CO_2 produced. The titration cup can be removed after incubation and solution be titrated directly without rinsing in order to avoid any solution spills (which may have happened in the Phase 1 titration). The flask was sealed with rubber bungs covered by aluminium foil and Vaseline gel to provide a better sealed system. No extra inoculum or nutrient was added as unnecessary (see discussion at Phase 1). After one-week incubation, NaOH titration was conducted immediately. Six 50 ml replicates of incubated samples were stored in

tubs without headspace until alkalinity measurement, which was carried out soon after NaOH titration.

Results: The titration results indicated 4.1 ± 0.13 mg C (in the form of CO_2) was captured by NaOH in the one-week incubation of 1 l stream water samples. The results in Table 3-6 showed the three-hour degassing by stirring removed 4.3 mg C l^{-1} free CO_2, with increasing sample pH and alkalinity. However as pH increased after degassing, the calculation predicted an increase in HCO_3^- content (from 27.9 ± 3.52 to 35.1 ± 0.37 mg CO_2 l^{-1}), with only little changes in CO_3^{2-}. Consequently, the degassing process led to only small amount of total DIC lost (2.6 ± 3.13 mg C l^{-1}).

Table 3-6 Total DIC loss after degassing and incubation. Concentrations of different forms of DIC were calculated using pH, alkalinity. Mean values (n = 6) are presented in the table. Numbers in brackets indicate the standard deviation.

Parameters	Before degassing	After degassing	After incubation
pH	6.2 *(0.03)*	6.5 *(0.06)*	6.8 *(0.09)*
Alkalinity (mg CO_2 l^{-1})	27.9 *(3.52)*	35.1 *(0.37)*	34.2 *(0.34)*
Free dissolved CO_2 (mg C l^{-1})	10.7 *(1.84)*	6.4 *(0.83)*	3.2 *(0.6)*
HCO_3^- (mg CO_2 l^{-1})	27.9 *(3.52)*	35.1 *(0.37)*	34.2 *(0.35)*
CO_3^{2-} (mg CO_2 l^{-1})	0.004 *(0)*	0.010 *(0.001)*	0.020 *(0.004)*
Total DIC loss (mg C l^{-1})	/	2.6 (3.13)	3.4 *(0.79)*

**Total DIC = free dissolved C-CO_2 + C-HCO_3^- + C-CO_3^{2-}

In total 3.4 ± 0.79 mg C l^{-1} of DIC was lost during this one-week incubation (mainly comprising loss from dissolved CO_2, 3.2 mg C l^{-1}). This loss from DIC pool likely contributed to the CO_2 captured by NaOH (4.1 ± 0.13 mg C l^{-1}), suggesting only a small BDOC decomposition (0.7 ± 0.99 mg l^{-1}) in these stream samples during the incubation.

In addition, a slight increase in pH occurred during incubation (from 6.5 ± 0.06 increased to 6.8 ± 0.09), and alkalinity slightly decreased (from 35.1 ± 0.37 to 34.2 ± 0.34 mg $CaCO_3$ l^{-1}). This contrasted with the observed changes during degassing before incubation, namely alkalinity increased with increasing pH.

Discussion: It was revealed that a large part of CO_2 dissolved in the NaOH solution was from DIC pool in water samples and not CO_2 generated by DOC respiration. Theoretically from the alkalinity and pH measurements with time, it should be possible to correct this DIC interference. However, the DIC measurement results were variable among replicates, which consequently led to large variation in BDOC, and so this approach of making a correction was not considered precise enough.

Another major concern was that pH increased in both occasions (degassing: from 6.2 to 6.5; incubation: from 6.5 to 6.8) which should theoretically lead to larger proportion of HCO_3^- fraction (Fig. 3-11). This only occurred in the degassing process, and not in the incubation period. This could not be explained exactly at this stage. One possibility might be that alkalinity was influenced by organic anions from humic materials (Cantrell et al., 1990; Barry et al., 2016).

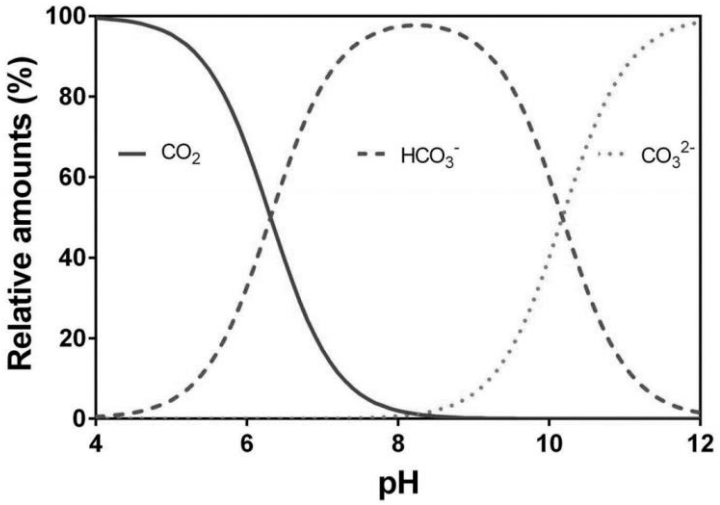


Fig. 3-11. Relative amounts of DIC fractions in water as a function of pH at 20 °C. The figure is adapted from (Pedersen et al., 2013).

Taken all together, CO_2 production measurement may overestimate BDOC loss, and it was not reliable to use alkalinity and pH measurements for the DIC interference correction. Thus CO_2 detection for estimating BDOC was not suitable for water incubation in this research.

3.2.2.3 Phase 3

Objectives: Phases 1 and 3 indicated that measurements of [DOC] changes and CO_2 production were both not precise enough to quantify the BDOC loss in Drumtee water samples. Therefore at Phase 3 another approach, measuring DO consumption (a common measurement for DOC biodegradation used by, e.g. Guillemette and del Giorgio, 2011; Moran et al., 1999), was tested to find out if it was a more precise method. In natural waters at dark, DO is only consumed by microorganisms to degrade DOC and not produced by photosynthesis, thus DOC consumption can be calculated from DO reduction.

Methods: In order to avoid interference of O_2 from atmosphere, the incubation has to be conducted in a properly sealed system without any headspace. As a result, smaller scale incubations systems were used to allow sacrificial sampling for frequent analysis.

Although this sealed system did not mimic the real aquatic system, it still provided significant information about potential biodegradability and DOC decomposition process and was the only way possible to stop DO exchange with the atmosphere. Thus it is commonly-used.

Bulk river water (2 l) was collected from the confluence site on August 26, 2015 and dispensed into six 250 ml brown reagent bottles without filtration once arrived in the lab. It was important to ensure that the bottles were fully filled with water and sealed completely. [DO] was measured in six sub-samples before incubation using a HQ10 Hach Portable LDO™ DO meter with luminescent sensor (a YSI DO meter with a membrane sensor based Clark electrode was also tested but showed less stability than the LDO one, thus was not used). The accuracy and resolution for the Hach DO meter were 0.1 mg l^{-1} and 0.01 mg l^{-1} respectively. According to the DO meter introduction, seven readings were taken for each sample and mean values were used in later calculation. DI water saturated in DO was used as standards before and in between [DO] measurements, to check the stability of DO meter.

The incubation was carried out without stirring in the dark room at 20 °C for one week. This time length was decided as just to examine if [DO] measurement was precise to detect BDOC loss. No extra inoculum or nutrient was added as unnecessary (see discussion at Phase 1). [DO] was measured again on the Day 7.

Results: Using changes in [DO] to estimate DOC degradation gave much more consistency between replicates and similar rates of loss compared to the other two approaches used earlier. Weekly BDOC decomposition was estimated as 0.77 ± 0.05 mg l^{-1} (Table 3-7).

Table 3-7 Biodegraded DOC loss in a one-week incubation indicated by DO consumption. Calculation is based on the equation C = 12*[DO]/32, according to which one mole of C mineralisation requires one mole of O_2. [DO] is the DO difference before and after incubation.

Replicate	DO (mg l^{-1})		DO consumption (mg l^{-1})	DOC biodegradation (mg l^{-1})
	Before incubation	After incubation		
WL13 1	9.05	6.99	2.06	0.77
WL13 2	9.06	6.84	2.22	0.83
WL13 3	9.06	7.19	1.87	0.70
WL13 4	9.06	7.03	2.03	0.76
WL13 5	9.07	6.91	2.16	0.81
WL13 6	9.06	7.06	2.00	0.75
Mean	9.06	7.00	2.06	0.77
SD	0.01	0.12	0.12	0.05

Discussion: The DO meter was sensitive enough (resolution as 0.01 mg l^{-1} O_2, namely 0.004 mg l^{-1} C) to detect the typically small changes in C degradation of this peaty water samples. A good stability and consistency was shown in this approach. In addition, it was also easier to have incubation conducted by using reagent bottles, without concerns about errors introduced from complicated procedures (e.g. titration and replacing NaOH containers) and the DO concentration was straight forward to measure.

Thus, considering the characteristics of my water samples (namely slow in biodegradation), and stability and reliability of all three methods tested, the [DO] measurement was chosen to be applied in the following incubation experiments to explore DOC biodegradation.

3.2.3 Summary of method development for biodegradation incubation experiment

This method development provided an overview of the slow BDOC decomposition in the Drumtee waters. Three commonly used measures for BDOC estimation were tested in this period. These experiments showed that the Thermalox TOC analyser was not precise enough to detect small changes in DOC in these humic water samples. Contribution from the existing DIC pool to CO_2 undermined estimating DOC degradation from CO_2 produced, and the interference cannot be properly corrected by measurements of alkalinity and pH. However DO consumption turned out to be an easy and stable method to quantify BDOC, detecting small measurement changes during the incubation, and thus was the approach chosen for the incubation experiments (Chapter 6).

Thus the protocol used was that untreated water samples would be incubated in individual reagent bottles with no headspace to avoid extra O_2. No nutrients or inoculum would be added (results from Phase 1). Samples would be measured sacrificially for [DO] at 3- or 4-day intervals to estimate BDOC loss. Although it was suggested at Phase 1 to carry out the incubation for 21 days. Due to the limited amount of DO in the 250 ml reagent bottles, there may be insufficient oxygen to support DOC respiration for up to 21 days. Exploration of the incubation length was undertaken in the first incubation experiment (Chapter 6).

4 Impacts of wind farm development on spatial and temporal variation of DOC quantity and quality

4.1 Abstract

This chapter considers a ten-year fluvial DOC concentration (2006 – 2016) time series and a two-year spectrophotometric composition (2014 – 2016) time series for the Whitelee catchments draining the south of Whitelee wind farm, to explore how wind farm disturbance influences DOC composition of the streams draining a peat-rich catchment. [DOC] across the Whitelee catchments showed an increasing trend during the ten years since the original wind farm development began and has not yet returned to the baseline level. [DOC] at sites WL13 and WL15, which were most affected by wind farm development, increased shortly after construction started, suggesting this activity causes increases in [DOC]. This interpretation of wind farm-related land use changes is further supported by the observed spatial and temporal variation of [DOC] between two tributaries within a small catchment Drumtee (5.7 km^2). The two tributaries are draining turbine-covered areas and felled forestry respectively. Similarly [SRP] increased at catchments WL13 and WL15 after construction, but no year-on-year increase was found. The macronutrient increase associated with wind farm construction is likely from the breakdown of organic matter e.g. from felled forestry. However, DOC export during the hydrological years 2011 – 2016 did not show a response concomitant with the increasing [DOC] trend indicating that controls on export of this macronutrient are more complex, and strongly influenced by climatic conditions (annual discharge).

Changes in DOC composition of receiving waters was also assessed at different scales: across the Whitelee catchments as well as between the two tributaries within Drumtee. The [Fe^{3+}]-corrected $SUVA_{254}$ and E_4/E_6 indicated broadly similar DOC aromaticity and FA:HA in HS pool across all Whitelee catchments. However the smaller $SUVA_{410}$ and larger E_2/E_4 in WL13 (Drumtee confluence) suggest there was a smaller HS proportion in WL13 DOC. While some climatic conditions would not differ much across the catchments, smaller [TON] and shorter water retention time in WL13 may contribute to the DOC composition difference.

In addition, land use may also influence the DOC composition. Within the Drumtee catchment the tributary draining the wind farm area exported more HS than the one draining felled forestry, indicated by greater $SUVA_{410}$ and smaller E_2/E_4. The results in the Drumtee catchment may be due to young DOC from the breakdown of residual branches

and roots in the felled area, or release of more humified organic matter from soils in the wind farm area as more deep peat was exposed due to peat excavation.

Furthermore hydrological conditions were observed to affect DOC composition both across the Whitelee catchments and between the tributaries. However in the Drumtee catchment, during the dry months DOC composition showed inverse changes between tributaries. This may be caused by the different terrestrial DOC sources from soil due to land use changes more obvious as the catchment became drier.

4.2 Introduction

It is estimated that a third of soil carbon in the world is stored in peatlands, which amounts to two-thirds of the atmospheric carbon pool (Limpens et al., 2008). The large amount of carbon stored may also be susceptible to changes in its release of gaseous carbon to atmosphere, or dissolved carbon to aquatic systems in response to global warming and changes in land use (Limpens et al., 2008). Land use change including deforestation, drainage and peatland burning, has been reported to facilitate releasing CO_2 to atmosphere (Hirano et al., 2009; Jauhiainen et al., 2005; van der Werf et al., 2009), and DOC from peat soils to stream water (Neal et al., 2004; Waldron et al., 2009), and it has been suggested C losses in peatlands should be associated with land use change (Hergoualc'H and Verchot, 2011). Land use is one of the factors contributing to significant vegetation change in terrestrial ecosystems, which can affect the carbon balance through its impacts on net primary productivity, the amount and ratio of CO_2:CH_4 emitted to the atmosphere (Limpens et al., 2008), and influence soil decomposition rates by altering the quantity and quality of litter inputs (Bardgett, 2005; Ward et al., 2015). Plant functional group removal, which happens in deforestation, has strong effects on soil biota and nutrient cycling associated with soil organic matter decomposition (Ward et al., 2015).

In Europe large areas of peat have been drained to improve grazing, lowering the water tables and stimulating DOC production (Worrall and Burt, 2004). [DOC] was observed to increase immediately during peat bog harvesting as a result of the ecosystem disturbance, but decrease afterwards, which might be due to a low content of stored DOM in the soil (Glatzel et al., 2003). Afforestation can lead to higher [DOC] for peat-covered catchments and forestry effects on [DOC] appear most significant following felling (Neal et al., 1998). Deforestation has both direct and indirect influences on C stocks and fluxes in terrestrial ecosystems (Bala et al., 2007). Timber-felling on peaty-mineral soils generally leads to an increase in stream water [DOC] which may persist for a few years, especially at a local scale (e.g. Neal et al. 2004). Forest clear-cutting has showed to significantly increase

[DOC] in boreal first-order streams (Schelker et al., 2014). Research on Asian tropical peat forests also measured significantly larger [DOC] for pore water in the deforested site than the pristine site, even without intentional drainage (Gandois et al. 2013).

However, whilst DOC concentration and fluxes associated with land use changes have been widely considered, not enough is known about how the composition of DOC responds to different land use management and how constant DOC composition will be. DOC composition and properties are significant in determining decomposition rate, which is responsible for the carbon release into atmosphere and determinative in terrestrial carbon storage potential (Jauhiainen et al., 2005; Ward et al., 2015). Compositional differences also indicates the origin of DOC (vegetation and soil leaching, microbial production) and reveal carbon cycling processes occurring in the peatland (Glatzel et al., 2003; Fellman et al., 2008; Gandois et al., 2013), and thus can also be affected by changes in balance during these processes. This understanding can inform further consideration of the fate of DOC in streams during transport, a key process in the global carbon cycle: terrestrial C moves laterally and is exported to the ocean or transformed to carbon dioxide where it can be degassed to the atmosphere (Long et al., 2015). As such it is important I consider the processes that control fluvial DOC composition and seek to understand if fluvial DOC composition varies in space and time.

Land use changes can be a key driver of DOC composition. As most fluvial DOC comes from the catchment soils, differences in fluvial DOC composition may reflect differences in the catchment soil reservoir, including how the land is managed. For example, afforestation in headwater streams can enrich the fulvic acid-like and protein-like DOC, which may be caused by the different plant litter qualities leading to different decomposition rates in the organic soil layer (Yamashita et al., 2011). Agriculture has been suggested to have changed the fluvial DOC to less terrestrial but more microbial-derived composition, and this may be due to the intensive usage of fertilizer, soil tillage and drainage changing microbial processing of DOC (Graeber et al., 2015).

A land use change challenge prevalent today is the disturbance of peatland soils for the construction of wind farms (Drew et al., 2013). Soil disturbance to bedrock and extensive deforestation can be required for wind farm construction and these activities may be expected to increase [DOC]. Indeed, short-term increases have been observed (e.g. Grieve & Gilvear 2008; Waldron et al. 2009). However only a few studies have considered the impact of wind power development on carbon cycling and export (Table 4-1). At the sub-catchment scale, ‘hot spots’ of high [DOC] (maximum of 113 mg C l^{-1}) were observed during construction in headwater streams draining a peatland in south west Scotland

(Niekerk, 2012). In Central Scotland [DOC] in streams draining wind farm site located in a blanket peat catchment was always larger than those in undisturbed stream in the period immediately after construction (Grieve and Gilvear, 2008). The additional DOC loss related to wind farm-induced disturbance was estimated at 5 g m^{-2}. Not only DOC, but also water chemistry, were found to be impacted by wind farm disturbance (Waldron et al., 2009). Consequently in receiving water, the changes in nutrient exports were suggested to support aquatic respiration rather than to increase microbial biomass, and thus greater CO_2 efflux could prevail.

Table 4-1 Key findings from existing research in the UK that has investigated the impacts on fluvial DOC from wind farm development on peatland.

Study site	Study period	Wind farm impacts on DOC	Source
Peat headwater hosting Whiteelee Wind Farm in south west Scotland	2006-2007	Wind farm -related disturbance resulted in temporal and spatial changes in stoichiometry between C, N and P of receiving waters.	(Waldron et al., 2009)
Blanket peat catchment in central Scotland	2006-2008	Greater [DOC] observed in wind farm-disturbed tributaries, with additional DOC loss.	(Grieve and Gilvear, 2008)
Peatland forest hosting Arecleoch Wind Farm in south Ayrshire, Scotland	2008-2010	The most disturbed catchment showed greatest [DOC]. Wind farm and/or forestry activities may result in extra DOC flux, increasing DOC concentration and humification. 'Hot spots' of high [DOC] was found inside the developed area.	(Niekerk, 2012)
Peat catchments hosting Whitelee Wind Farm in south west Scotland	2007-2010	Percentage of peat-based soil has influence on streamwater C, and wind farm-related disturbances were found to control streamwater dynamics. Forestry operations most likely resulted in additional C and P in stream.	(Murray, 2012)
Peat catchment hosting Gordonbush Wind Farm in North East Scotland	2010-2014	Wind farm-related forest felling and peat coverage may contribute to greater [DOC]. Construction activities may result in an increase in aquatic C export.	(Smith, 2016)
Peat catchments hosting Whitelee Wind Farm extension in south west Scotland	2011-2013	Forest felling was found to be significantly positively correlated with DOC concentration and export, while tracks, cable trenches and turbines exerted negative control. [DOC] increased during wind farm development and took five years to recover.	(Phin 2015)

Long-term monitoring is suggested for assessments of wind farm development. In Whitelee, UK's largest onshore wind farm, a ten-year monitoring has been conducted through a series of research projects since the original construction. An increase in [DOC] was measured during the windfarm construction in the catchment closest to the Whitelee wind farm, with the highest DOC export in the year after construction started (Murray, 2012). Furthermore this increasing impact was continuously monitored and approximately five years following development [DOC] was suggested to return to pre-construction levels (Phin 2015). Consistent in several studies was that wind farm-related forestry operations e.g. clear-felling, was considered most likely to impact aquatic carbon concentration and/or export (Table 4-1).

Within the limited research, the impacts of wind farm development on terrestrial C loss to aquatic systems has mainly focused on quantifying DOC loss, with only one study assessing DOC composition, specifically humification using E_4/E_6 (Niekerk, 2012). Thus little is known about whether the increased DOC exported to streams becomes more resistant or susceptible to decomposition, yet this information could be useful to understand the source of released DOC and what controls this. Here in addition to understand the wind farm impacts on water chemistry and quality by continuing the long-term in-stream [DOC] monitoring, DOC composition was measured not only at the catchment-scale, but also within one catchment previously most impacted by wind farm development. Further, drawing on the compositional characteristics of different DOC pools (HS, HAs and FAs) described earlier (Chapter 1), UV spectrophotometric methods and parameters were used to assess DOC composition and to explore:

1) how long wind farm-associated disturbance can influence water chemistry in peaty catchments, by analysing mainly the trends of DOC concentration and export before and after wind farm development started; variations of [TON] and [SRP] were also considered;

2) if wind farm-related land use differences (mainly wind farm construction and deforestation) caused any spatial difference in [DOC] between catchments experienced wind farm disturbance, as well as two tributaries in a small catchment;

3) whether this released DOC differed in composition and how is the variation related to the two specific land uses.

4.3 Methods and materials

4.3.1 Water sampling strategy

In order to understand the long-term impacts of wind farm-related disturbance on peatland catchment water, stream samples for DOC, SRP and TON were collected from the outlets of the five Whitelee catchments (WL13, 14, 15, 1 and 16), which drain the south of the Whitelee windfarm, approximately every four weeks from April 2014 – September 2016. This continued a sampling programme which started in July 2006, but augmented it by considering also DOC composition. Additionally, on the same day as long-term water sampling in 2014 – 2015, water samples were collected from the Drumtee Catchment (WL13) in order to investigate over one year the DOC concentration and composition: eight sites on the stream draining the wind farm area (Stream D-WF) and seven sites on the other draining the felled forestry (Stream D-FF) (Fig. 4-1, modified and reproduced from Fig. 2-5 for ease of reading). Full information about the field sites and sampling procedures can be found in Chapter 2.

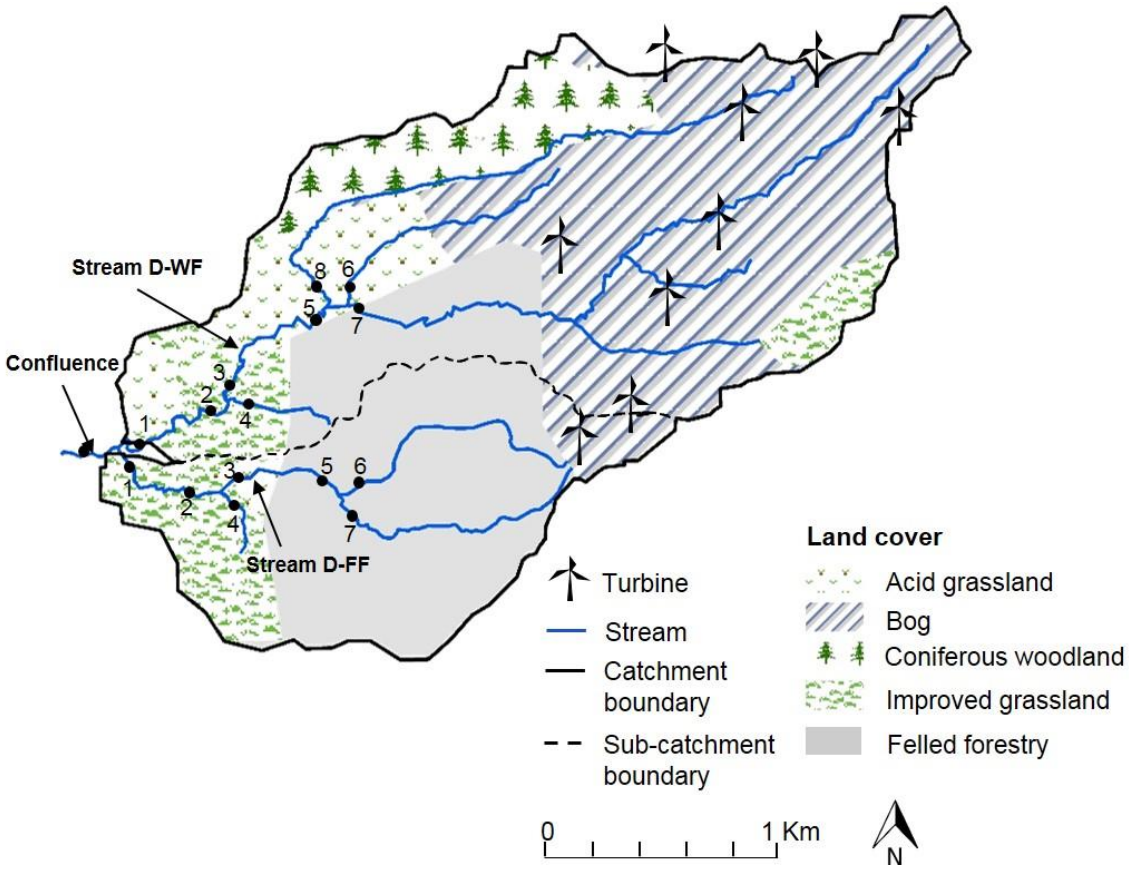


Fig. 4-1. Sampling points for short-term DOC analysis (February 2014 – February 2015) in the Drumtee Catchment. Land use data was from Digimap® Land Cover Map 2015.

4.3.2 Water sample quantity and quality analysis

Both long-term and short-term water samples were analysed for [DOC] and UV-visible absorbance. Optical parameters ($SUVA_{254}$, $SUVA_{410}$, E_2/E_4 and E_4/E_6) were calculated after absorbance at 254 nm was corrected for Fe^{3+} interference. For long-term samples, [SRP] and [TON] were also measured. The procedures for sample collection, treatment, measurement and calculation of the above parameters are detailed in Chapters 2 and 3.

4.3.3 Annual specific DOC flux calculation

Stream water discharge at Drumtee confluence site during 2011 - 2016 was scaled to catchment area for other Whitelee catchments (WL14, 15, 16 and 1) upon the assumption that specific discharge was similar in these adjacent catchments. Annual DOC flux was estimated according to 'Method 5' for each hydrological year from Oct. 2011 – Sept. 2016, and divided by the catchment area to generate specific annual DOC flux. Details are described in Chapter 2, Section 2.4.2.

4.3.4 Data analysis

In Drumtee, temporal variation of sub-catchment was characterised by the mean values and the standard deviation of all sampling points for each sampling trip. This was undertaken to provide more confidence that the variation in each sub-catchment composition was captured in the single monthly average. This approach has a degree of pseudoreplication. However, around 50 % of the sampling points receive no water from upstream and thus were independent of one another, and all sites received new water.

For Drumtee samples, one way analysis of variance (ANOVA) of the pooled data was undertaken to explore if there were significant inter-stream differences for all DOM optical parameters. Among the five long-term-monitored Whitlee catchments, ANOVA was also used to examine inter-catchment variation during 2014 – 2016 for the DOM monthly optical parameters. Before any ANOVA analysis, the Anderson-Darling method was applied to examine the normality of each dataset and all datasets in this study (including both long- and short-term data) were normally distributed. A Tukey Test in ANOVA was used. All normality and ANOVA analyses were conducted using Minitab® 17 statistical software. A p value < 0.05 was considered as significantly different.

The Seasonal Kendall test (Hirsch et al., 1982; Hirsch and Slack, 1984) was used to analyse nonparametric seasonal changing patterns in [DOC], [SRP] and [TON]. This test for trend was further developed by the U.S. Geological Survey for a computer programme and has

become the most frequently used test for trend in the environmental sciences (Helsel et al., 2006). In this research, the Seasonal Kendall test (Type 2) was applied and where the slope value > 0 means an increasing trend (and vice versa), and $p < 0.05$ means the change is significant. Based on the windfarm activities, the time series (July 2006 – September 2016) can be divided into periods: July 2006 – October 2010 for the first windfarm development and following two years afterwards, and November 2010 – September 2016 for the extension activities and four years afterwards. The Seasonal Kendall test were applied for the complete time series and the two divided groups.

4.4 Results

4.4.1 Stream [DOC] time series (2006 – 2016) in the Whitelee catchments

A seasonal cycle for [DOC] was observed in all sites and for the duration of the 10-year time series (Fig. 4-2), with the largest concentration in the summer periods (July – October) and the smallest in the winter and spring (November – April). The maximum [DOC] was 66.8 mg C l^{-1} in WL15 (July 2016) and the minimum was 5.2 mg C l^{-1} in WL1 (February 2007). WL15 had the greatest median [DOC] (30.7 mg C l^{-1}) during these years, followed by WL13 (26.9 mg C l^{-1}) (Table 4-2). The concentrations were similar between sites in most months through all the years, but in the summer period there were relatively higher [DOC] in WL13 and WL15, which are sites closest to the original and extension wind farm development respectively.

The maximum and median [DOC] at WL13 showed the greatest increase from HY2006-07 to HY2007-08 (maximum: from 40.9 to 57.1 mg C l^{-1}, median: 18.1 to 29.8 mg C l^{-1}), but for the same time period only slight increases were shown at most of other sites (Fig. 4-2 and Fig. 4-3). During the first phase of windfarm development (October 2006 – May 2009), the highest [DOC] of the five sites was constantly observed in WL13 (Fig. 4-2). At all sites a higher [DOC] (maximum, median and minimum) than the value during HY2006 – 2007 was found in HY2009-10, one year after the original development was finished (Fig. 4-3). Furthermore, during July 2006 – Oct. 2010 (the original development and two years afterwards), significant increases were found for all site ($p < 0.05$, Table 4-2).

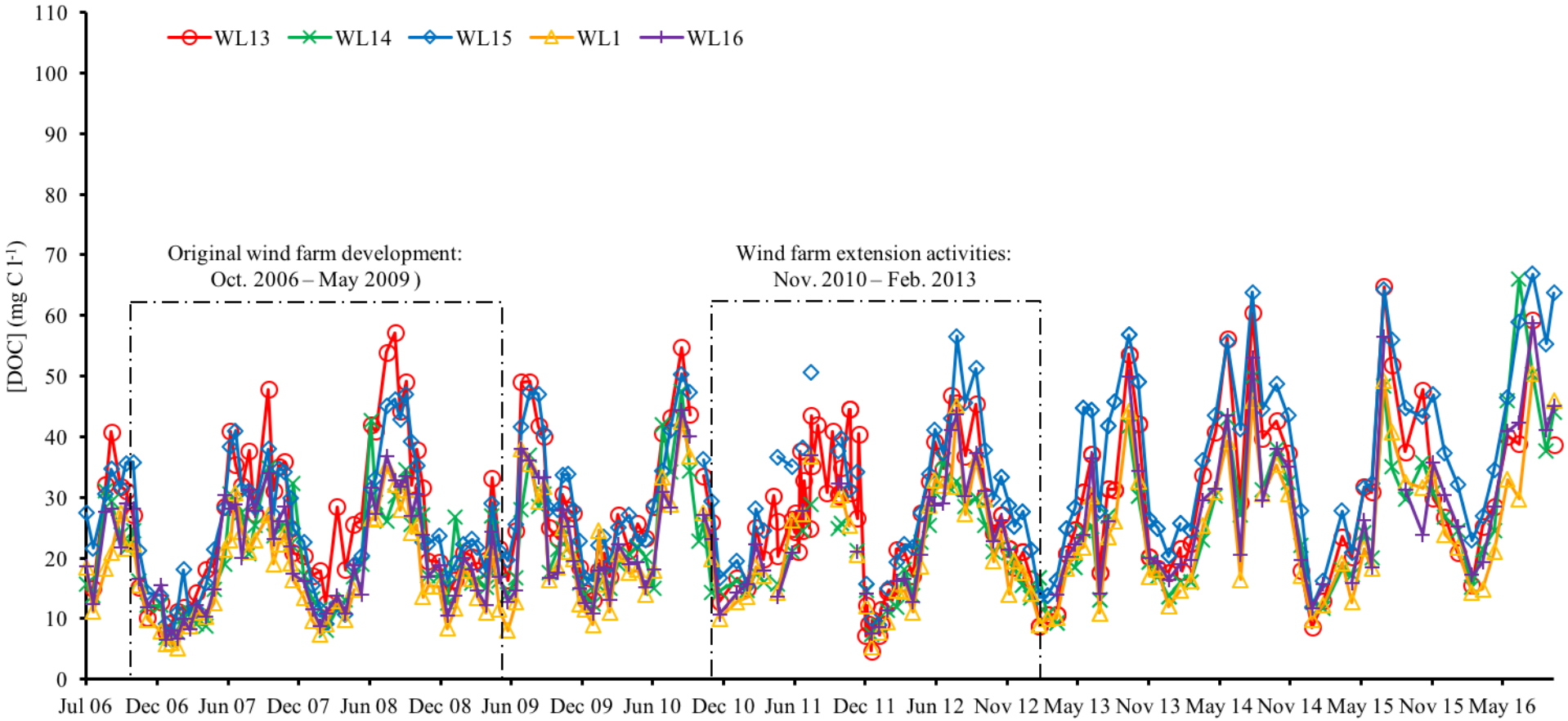


Fig. 4-2. Long-term time series (July 2006 – Sept. 2016) of [DOC] for catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Dash boxes indicate time periods of original windfarm development (all catchments affected, Oct. 2006 – May 2009), and extension (WL15 affected most, Nov. 2010 – Feb. 2013). Data prior to Aug. 2011 was collected by Murray (2012) and Waldron et al. (2009), and between Sept. 2011 – Mar. 2013 by Phin (2015).

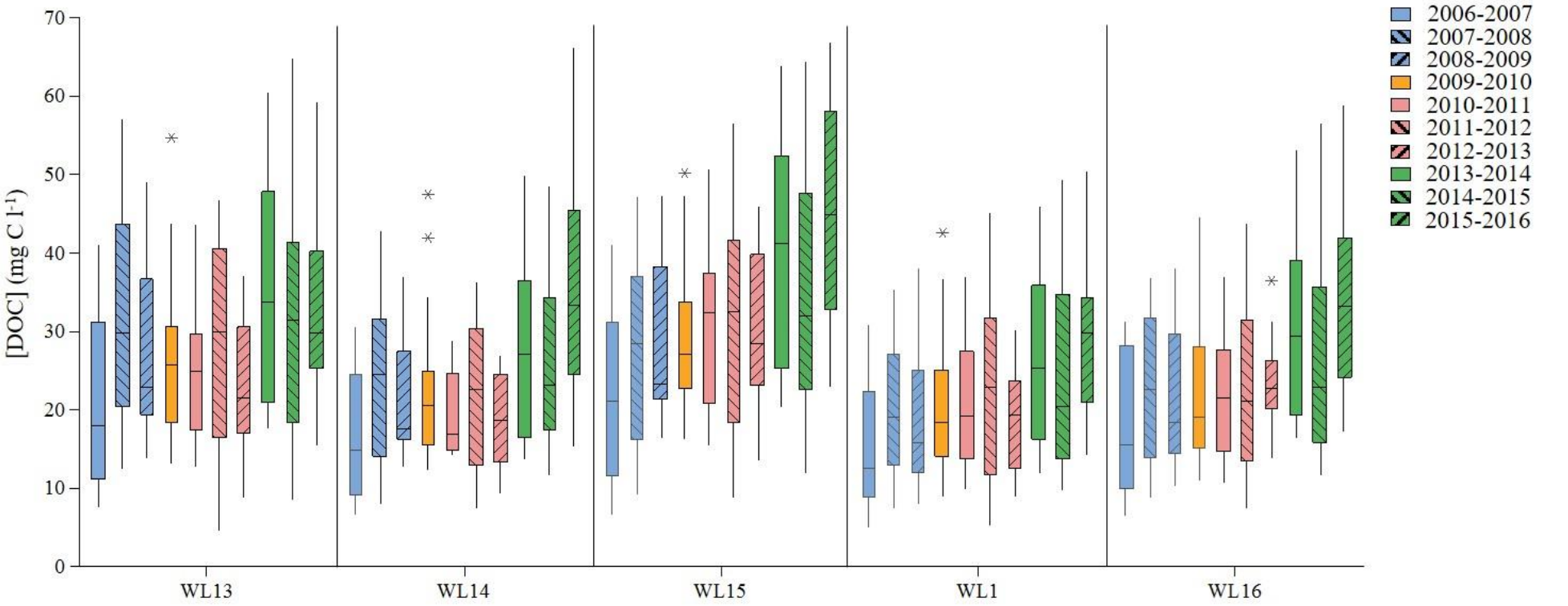


Fig. 4-3. Box-plots showing Long-term time series (Oct. 2006 – Sept. 2016) of [DOC] for catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Each coloured bin is pooled data for a hydrological year and it is noted that 2015-2016 is not a full year. The boxed area shows interquartile range, the median is the horizontal line within the box, and the vertical lines extending from the boxes show the minimum and maximum. Data point outliers are shown as asterisks. Data prior to Aug. 2011 was collected by Murray (2012) and Waldron et al. (2009), and between Sept. 2011 – Mar. 2013 by Phin (2015).

Table 4-2 Median values, p values, significance and trend slope for [DOC] in catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Data was grouped into three: the total 10 years, July 2006 – Oct. 2010 for the original wind farm development and two years afterwards, and Nov. 2010 – Sept. 2016 for the extension and three years afterwards. Numbers in bold red are indicating the largest trend slopes for significant changes. * p < 0.05, ** p < 0.01, *** p < 0.001.

Years	Sites	Median [DOC] (mg l^{-1})	p	Significance	Slope
July 2006 – Sept. 2016	WL13	26.9	0.022	*	1.12
	WL14	22.2	0.011	*	1.17
	WL15	30.7	0.003	**	**1.91**
	WL16	22.8	0.003	**	1.30
	WL1	20.6	0.004	**	1.26
July 2006 – Oct. 2010	WL13	26.0	0.008	**	**2.63**
	WL14	20.9	0.032	*	2.54
	WL15	25.5	0.000	***	2.58
	WL16	19.8	0.005	**	2.04
	WL1	17.9	0.000	***	**2.63**
Nov. 2010 – Sept. 2016	WL13	27.3	0.001	***	1.71
	WL14	24.3	0.006	**	2.13
	WL15	34.9	0.004	**	**2.38**
	WL16	25.3	0.007	**	1.98
	WL1	21.5	0.018	*	1.47

However, from 2011 the greatest [DOC] of the five sites was observed in WL15 (Fig. 4-2), which was the catchment most affected by the extension. Noticeably, during HY2010 - 11 (the extension year), when maximum and median [DOC] were constant or decreased for most sites compared to HY2009 - 10, increases of both values were observed at WL15 (maximum: from 47.2 to 50.7 mg C l-1, median: 27.0 to 32.2 mg C l^{-1}, Fig. 4-3). The high [DOC] in WL15 did not reduce after the windfarm extension was completed. Furthermore, over the extension period and the following years (Nov. 2010 – Sept. 2016), significant increasing trends of [DOC] were observed at all sites (although a decrease occurred during 2012 – 2013) ($p < 0.05$, Table 4-2, Fig. 4-3). WL15 presented the largest increasing slope compared to other sites (Table 4-2). Over the 10 years significant increasing trends were suggested for all five sites ($p < 0.05$, Table 4-2), with the largest slope at WL15, indicating [DOC] has been increasing the fastest.

Taken all together, greater [DOC] and the greatest increase were observed in WL13 during the original wind farm development. However, [DOC] in WL15, which hosted most of the extended turbines, showed the greatest [DOC] since the extension began.

4.4.2 Annual specific DOC export from the Whitelee catchments

The annual specific DOC exports from the Whitelee catchments were calculated for hydrological years 2011 – 2016, when the stream discharge data at WL13 is available. As in 2016 the discharge monitoring was stopped in April and low discharge in the drier periods were not recorded, there may be an overestimate of the annual discharge in this year.

Table 4-3 Annual specific DOC export (g m^{-2} yr^{-1}) from the Whitelee catchments during Oct. 2011 – Apr. 2016. It should be noted 2016 did not have data for a complete hydrological year (recording stopped in April 2016).

Hydrological year	WL13	WL14	WL15	WL1	WL16
2011-2012	36.3	27.3	39.2	29.5	31.8
2012-2013	26.0	20.8	33.4	22.4	17.3
2013-2014	47.1	37.2	51.9	36.6	41.0
2014-2015	22.4	17.6	23.4	16.6	13.6
2015-2016	25.5	23.1	34.9	25.1	26.5

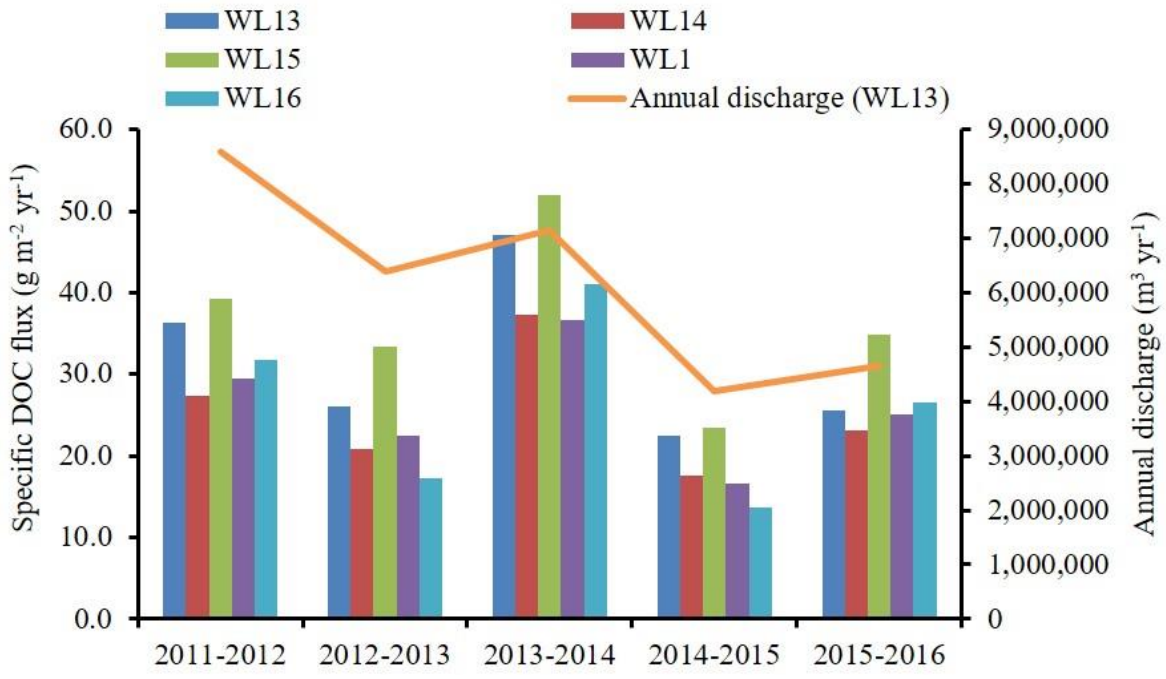


Fig. 4-4. Annual specific DOC export from the Whitelee catchments from Oct. 2011 – Apr. 2016. It should be noted 2016 did not have data for a complete hydrological year. The hydrological year total discharge measured at WL13 is represented by the orange line.

The annual DOC exports ranged from 13.6 g m^{-1} yr^{-1} (at WL16 during 2014 – 2015) to 51.9 g m^{-2} yr^{-1} (at WL15 during HY2013-14) (Table 4-3 and Fig. 4-4). There was a similar

temporal trend of DOC export in each of the catchment, with the greatest fluxes occurring in HY2013 - 14 (36.6 – 51.9 g m^{-2} yr^{-1} across catchments), followed by those during HY2011 - 12 (27.3 – 39.2 g m^{-2} yr^{-1}). The smallest fluxes were observed in HY2014 – 15 (13.6 – 23.4 g m^{-2} yr^{-1}), about one year after the extension activities finished. This temporal trend in DOC flux is broadly similar to the variation in annual stream water discharge.

In all years WL15 (23.4 – 51.9 g m^{-2} yr^{-1}) had the greatest DOC export between catchments, followed by WL13 (22.4 – 47.1 g m^{-2} yr^{-1}). Both had WL13 and WL15 had larger [DOC] than the other catchments (Fig. 4-2).

4.4.3 Seasonal [DOC] (2014 – 2015) in Drumtee Catchment

Drumtee represents the long-term site WL13 of more intense monitoring (e.g. Coleman, 2017) and so this site was chosen for one year to consider also the sub-catchment spatial variation in DOC concentration and composition, related to the land use. Across the two tributaries and confluence in the Drumtee Catchment, [DOC] ranged from 5.2 to 89.3 mg C l^{-1} from February 2014 – February 2015, with the largest [DOC] for D-FF6 in August 2014 and the smallest for D-WF6 in January 2015(Fig. 4-5 upper).

All tributary [DOC] exhibited little intra-stream variation, apart from site D-WF4 (represented by the green line in Fig. 4-5 upper), which had concentrations more similar to D-FF group. This site, unlike others in D-WF, had its source within the felled area. As this section focuses on the comparison between the two sub-catchments, to explore if land-use affects DOM composition, it was considered more appropriate to pool this data with the D-FF samples. Doing this reduces intra-stream variation and the more similar tributary group composition facilitates consideration of inter-stream difference. The clarification for this decision (moving D-WF4 to D-FF group) is further detailed in Appendix A – Drumtee data treatment. This re-grouping method is therefore applied to the consideration of both DOC concentration and composition in Drumtee, and the primary data are provided in the sections 4.4.3 and 4.4.5 to allow the comparison between D-WF and D-FF groups.

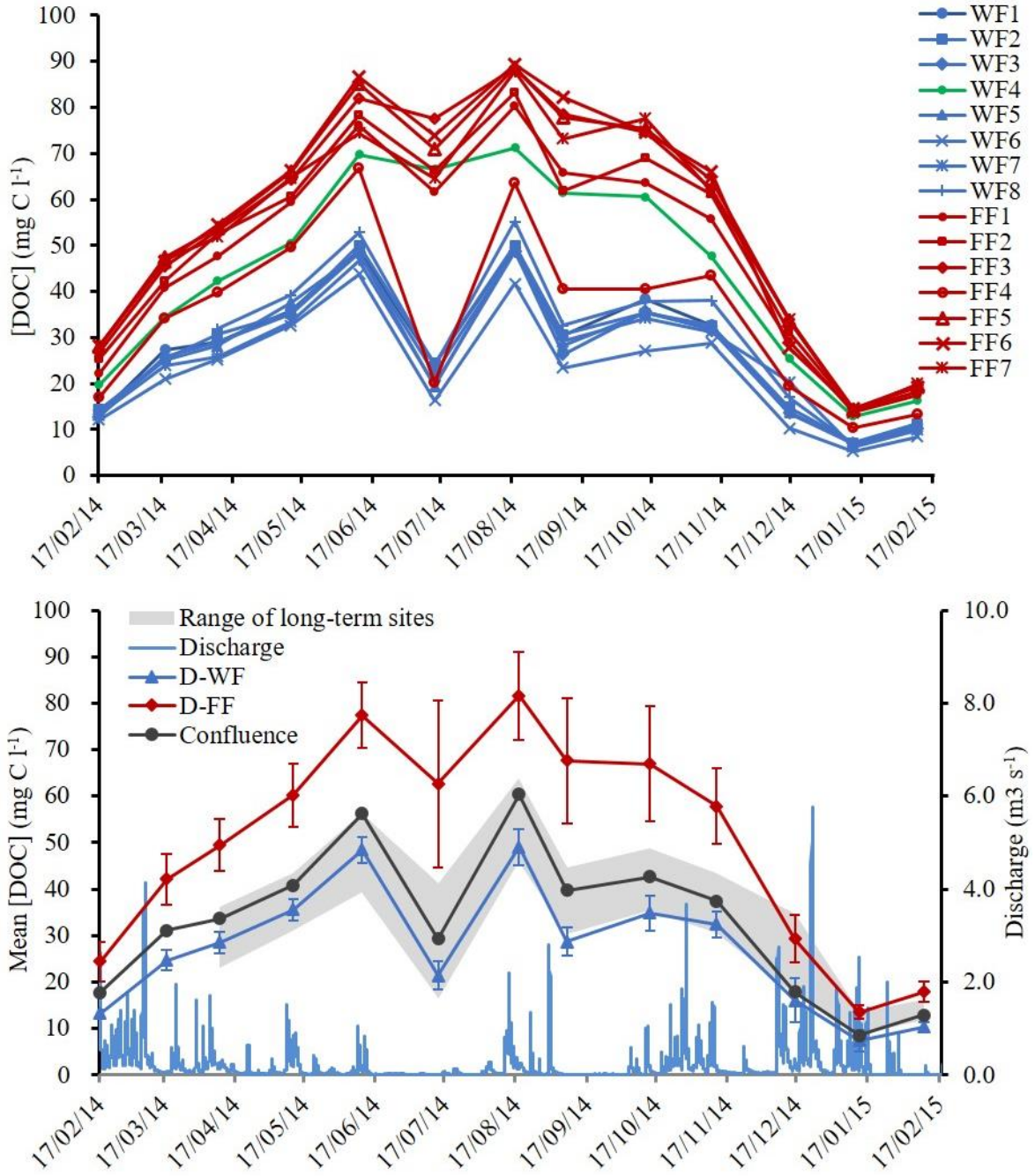


Fig. 4-5. [DOC] of all sampling sites along the Drumtee tributaries (Fig. 2-1) for each month from February 2014 to February 2015 were shown in the upper panel. The mean values of each tributary and the confluence water are shown in the lower panel, where each sample point is the mean ± SD of all intra-stream points sampled on the same day. D-WF4 was pooled with D-FF. The grey area shows the range of [DOC] in the five Whitelee catchments.

After re-grouping, more obvious differences were observed between streams (Fig. 4-5 lower). The range in [DOC] in both streams was large, with D-FF having greater monthly mean [DOC], ranging from 13.5 ± 1.4 – 81.6 ± 9.5 mg C l^{-1} across the year, compared to from 6.6 ± 0.7 – 49.0 ± 4.0 mg l^{-1} for D-WF. Monthly [DOC] in the confluence water was in between the two tributaries, but closer to D-WF, ranging from 8.6 – 60.4 mg l^{-1}. In addition, monthly D-WF [DOC] was similar to Whitelee long-term sites and towards the lower end

of the range, whilst D-FF showed [DOC] towards the higher end of the range (Fig. 4-5 lower).

Generally, [DOC] in the Drumtee tributaries showed similar seasonal patterns to that observed in long-term sites, with the lowest concentrations in winter and highest in summer. However, there were [DOC] decreases across Drumtee in both July and September, which were the dry periods in 2014 (Fig. 4-5 lower). Similar decreases were observed for [DOC] in the long-term sites as well. There were smaller decreases in D-FF in September (of 14.6 mg C l^{-1} in July and 14.0 mg C l^{-1}) than in D-WF (of 27.1 mg C l^{-1} in July and 20.3 mg C l^{-1}).

4.4.4 DOC composition in the Whitelee catchments

$SUVA_{254}$ in all long-term sites ranged from 2.2 – 4.2 l mg^{-1} m^{-1} between April 2014 to September 2016, with the smallest in WL1 (October 2015), and the largest in WL15 (January 2015) (Fig. 4-6). The mean values of monthly $SUVA_{254}$ for all sites had a small range from 3.4 ± 0.4 – 3.5 ± 0.4 l mg^{-1} m^{-1} (Table 4-4). The range of $SUVA_{254}$ for Whitelee was within the range observed from other samples from different aquatic systems, with the mean values towards the higher end of range (Table 4-5). Mean $SUVA_{254}$ for long-term sites was similar to that in other peaty waters, such as a Canadian peatland well water (Olefeldt et al., 2013). No significant difference was measured for $SUVA_{254}$ among sites ($p > 0.05$, Table 4-4). All $SUVA_{254}$ varied throughout the observation period (Fig. 4-6). There was no seasonal pattern in $SUVA_{254}$, but a decrease was observed in October 2015 and then a gradual increase towards the summer months, although variable.

$SUVA_{410}$ in all Whitelee sites ranged from 0.32 – 0.63 l mg^{-1} m^{-1}, with the largest in WL16 in September 2014 and the smallest in WL13 in October 2015 (Fig. 4-6). WL13 also had the smallest monthly mean $SUVA_{410}$ (0.47 ± 0.05 l mg^{-1} m^{-1}) compared to other sites (Table 4-4). ANOVA analysis indicates monthly mean $SUVA_{410}$ in WL13 was significantly different from that in all the other sites bar WL14. Smaller $SUVA_{410}$ for WL13 indicates less complex aromatic carbon in the DOC. No seasonal pattern was observed for $SUVA_{410}$ but it showed a similar temporal trend to that of $SUVA_{254}$, with an obvious decrease in October 2015. It was also noticed in January 2016 (a cold and wet period) $SUVA_{254}$ and $SUVA_{410}$ increased in WL13 but decreased in the other catchments.

Although both SUVA parameters varied throughout the years, in summer and autumn DOC seemed to be less aromatic after a dry period. For example, both SUVA decreased in August 2014 and October 2015, Fig. 4-6).

Table 4-4 Mean values of monthly optical parameters ($SUVA_{254}$, $SUVA_{410}$, E_2/E_4 and E_4/E_6) of the long-term sites (Fig. 2-1) from 2014 - 2016. The difference was examined using ANOVA, and $p < 0.05$ suggests there was significant difference within the group. Where one site is different to the others this is indicated in bold.

	$SUVA_{254}$ ($l\ mg^{-1}\ m^{-1}$, p = 0.739)	$SUVA_{410}$ ($l\ mg^{-1}\ m^{-1}$, p = 0.007)	E_2/E_4 (p = 0.000)	E_4/E_6 (p = 0.128)
WL13	3.4 ± 0.4	**0.47 ± 0.05***	**7.3 ± 0.5**	7.3 ± 1.1
WL14	3.4 ± 0.4	0.49 ± 0.05	7.0 ± 0.3	6.8 ± 0.5
WL15	3.5 ± 0.4	0.51 ± 0.05	6.9 ± 0.4	7.1 ± 0.7
WL16	3.5 ± 0.3	0.52 ± 0.05	6.7 ± 0.5	7.4 ± 1.2
WL1	3.4 ± 0.4	0.50 ± 0.05	6.7 ± 0.4	7.3 ± 1.1
Implication	Similar DOC aromaticity	Less contents of more complex aromatic C in WL13	Smaller proportion of HS in WL13 DOC	Similar FA:HA

*WL13 $SUVA_{410}$ was significantly smaller than all the other sites bar WL14.

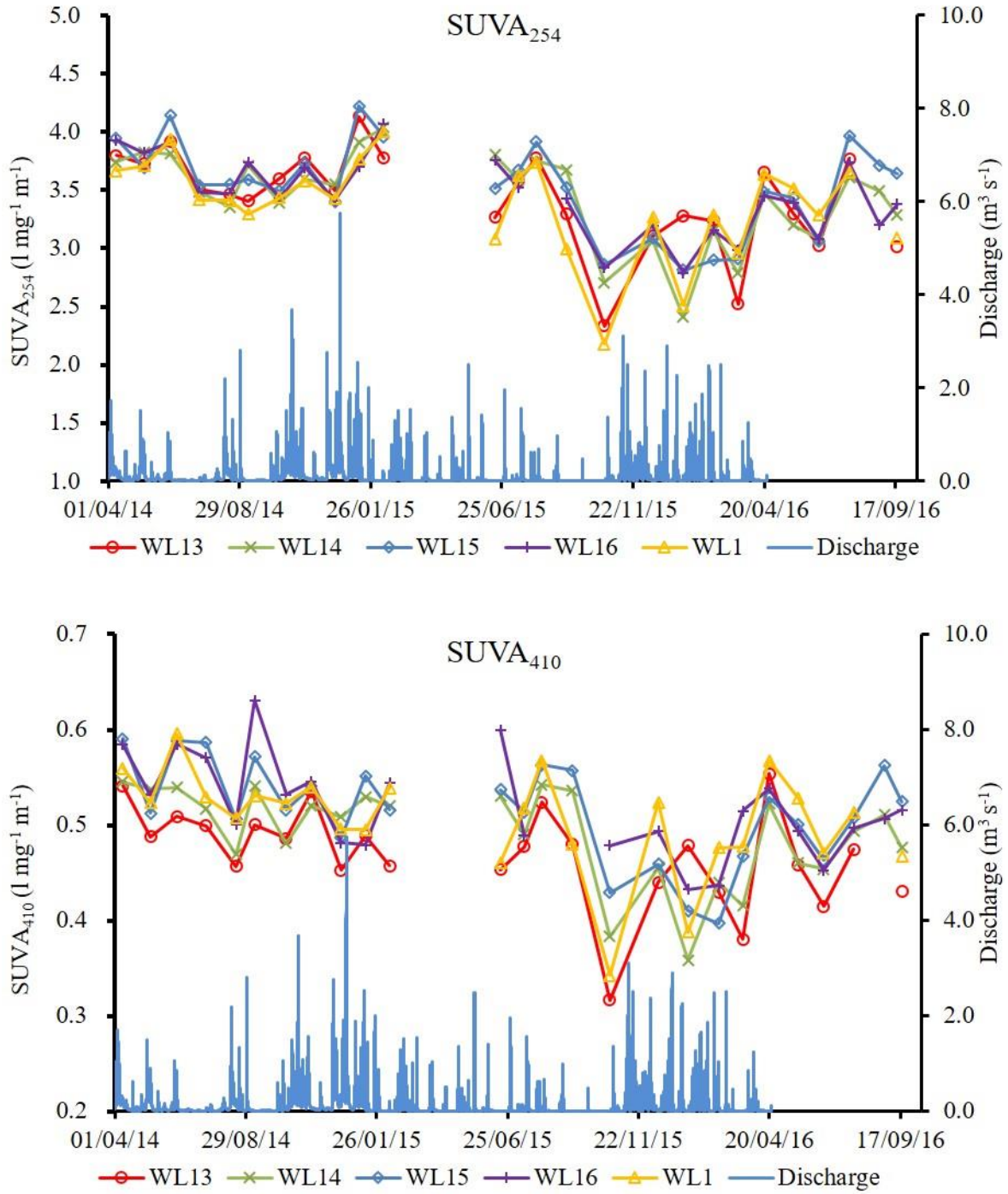


Fig. 4-6. $SUVA_{254}$ (upper) and $SUVA_{410}$ (lower) for DOC in the five long-term sites (Fig. 2-1), from April 2014 – September 2016. DOC composition of the long-term sites was only measured since April 2014. No UV data was measured during March to May 2015 due to indecisive research plan. All UV absorbance was corrected for Fe^{3+} interference. Discharge recorded in WL13 was used to indicate flow conditions in all sites. Due to construction in the site, discharge recording stopped after 22 April 2016.

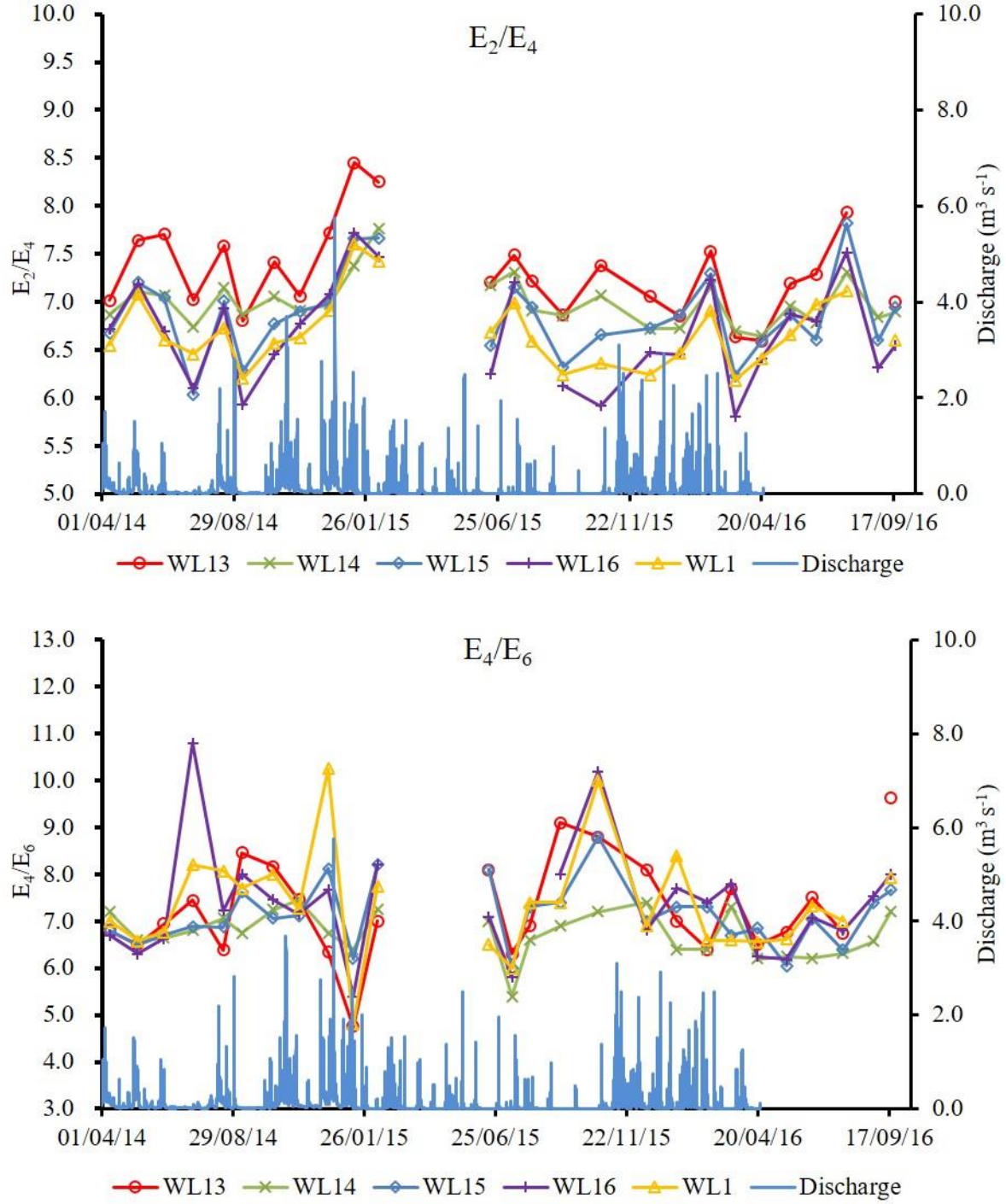


Fig. 4-7. E_2/E_4 (upper) and E_4/E_6 (lower) for DOC in the five long-term sites (Fig. 2-1), from April 2014 – September 2016. DOC composition of the long-term sites was only measured since April 2014. No UV data was measured during March to May 2015 due to indecisive research plan. All UV absorbance was corrected for Fe^{3+} interference. Discharge recorded in WL13 was used to indicate flow conditions in all sites. Due to construction in the site, discharge recording was stopped after 22 April 2016.

E_2/E_4 in all Whitelee sites ranged from 5.8 – 8.4 across the years. E_2/E_4 of WL13 was constantly higher than the rest sites all through the years (Fig. 4-7), and the average monthly value was measured to be significantly different from others (Table 4-4). A larger E_2/E_4 represents either greater total aromatic C content (Abs_{254}), or less of the complex aromatic C (Abs_{410}). Here E_2/E_4 indicates proportionally less complex aromatic C (HS) in WL13, as $SUVA_{254}$ was not significantly different among sites. E_2/E_4 in all sites varied temporally but showed a similar seasonal trend. The increase in E_2/E_4 in all sites respectively in 2014 winter (cold and wet) and 2016 summer (warm and dry) suggests proportionally less HS in DOC.

E_4/E_6 ranged from 4.8 (WL13, January 2015) to 10.8 (WL16, July 2014) through the period but was more spatially variable than E_2/E_4 (Fig. 4-7). The mean monthly E_4/E_6 ranged from 6.8 ± 0.5 – 7.4 ± 1.2 between the five sites, but no statistically significant difference was suggested across sites ($p > 0.05$, Table 4-4). No seasonal pattern was found for any site. However, it was noted that in January 2015 E_4/E_6 decreased for all sites, and at the same time E_2/E_4 increased. Together these may indicate the DOC in that month had proportionally less HS than non-humic materials than earlier (shown by the increased E_2/E_4). However the HS became more humified (smaller FA:HA, shown by the decreased E_4/E_6) and thus resistant. An increase of E_4/E_6 in October 2015 was observed for most sites, indicating DOC became less humic. This was in the agreement with decreased SUVA and increased E_2/E_4 in the same month (Fig. 4-6 and Fig. 4-7).

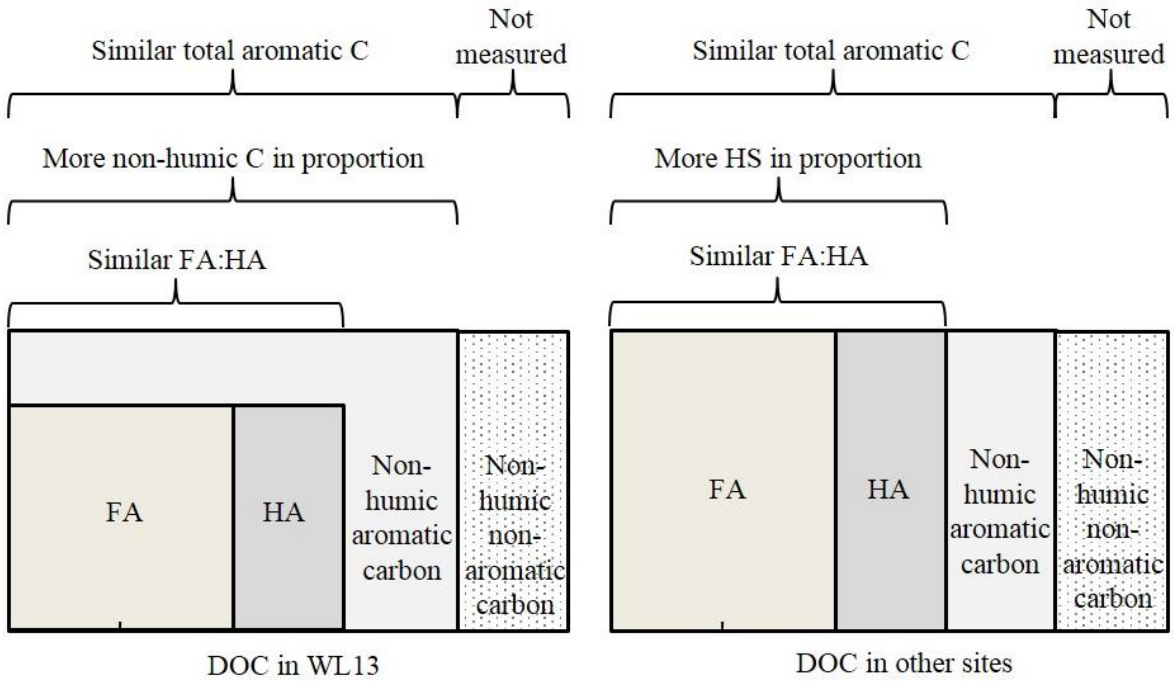


Fig. 4-8. Schematics showing the different proportions of HS in DOC produced from WL13 and the other four Whitelee catchments, with the relative areas hypothesised to represent.

The differences in DOC composition between WL13 and the other catchments as indicated by the spectrophotometric composition can be summarised (Fig. 4-8), driven primarily by differences in $SUVA_{410}$ and E_2/E_4 (Table 4-4). In summary these indicate that WL13 was less humified, and contained a greater proportion of non-humic materials, but similar FA:HA of humic substance pool.

4.4.5 DOC composition in the Drumtee Catchment

Monthly mean $SUVA_{254}$ in the Drumtee catchment along two streams ranged from 2.8 ± 0.3 to 4.5 ± 0.3 l mg^{-1} m^{-1}. For most months $SUVA_{254}$ in Drumtee was close to the range of Whitelee long-term sites (Fig. 4-9). The annual mean value of $SUVA_{254}$ across Drumtee (3.7 ± 0.32 l mg^{-1} m^{-1}) was within the range of diverse surface freshwater (Table 4-5), but towards the higher end, indicating the fluvial DOC in Drumtee catchment was rich in aromatic carbon. Generally, throughout the year DOC in the two tributaries had similar aromaticity with small variation (Fig. 4-9). The annual mean $SUVA_{254}$ between two streams was suggested to be not significantly different ($p > 0.05$, Table 4-5). However different responses in streams were recognized in summer and winter respectively. The D-WF DOC aromaticity decreased clearly from 3.9 ± 0.0 to 2.8 ± 0.3 l mg^{-1} m^{-1} in July 2014 (the dry month) when there was a slight increase in D-FF (from 3.7 ± 0.1 up to 3.8 ± 0.3 l mg^{-1} m^{-1}). Moreover, during the wet winter period, D-WF DOC aromaticity increased in January 2015 (from 3.6 ± 0.1 up to 4.5 ± 0.3 l mg^{-1} m^{-1}), before decreasing in the following month (Fig. 4-9), while it was less variable in the other stream.

Most months, $SUVA_{410}$ was higher in D-WF (0.39 ± 0.07 – 0.56 ± 0.03 $lmg^{-1}m^{-1}$) than D-FF (0.38 ± 0.04 – 0.50 ± 0.01 $lmg^{-1}m^{-1}$) (Fig. 4-10), and all the values are close to those of peatland waters (Lou et al., 2014, Table 4-5). In most months, $SUVA_{410}$ of D-WF was close to the lower end of the Whitelee range. The significantly larger mean annual $SUVA_{410}$ in D-WF DOC (0.50 ± 0.05 $lmg^{-1}m^{-1}$) than D-FF (0.45 ± 0.04 $lmg^{-1}m^{-1}$) ($p < 0.05$) indicates the greater amount of complex aromatic materials in D-WF DOC. Both tributaries had $SUVA_{410}$ smaller than that in long-term sites, and $SUVA_{410}$ for D-WF was close to the lower edge of long-term range. Generally, there is no clear seasonal pattern in both streams. However, similar to changes in $SUVA_{254}$, there was a decrease for D-WF $SUVA_{410}$ in July (from 0.53 ± 0.01 to 0.39 ± 0.07 l mg^{-1} m^{-1}) and an increase for D-FF (from 0.45 ± 0.03 to 0.49 ± 0.06 l mg^{-1} m^{-1}). The standard deviations of the data in this month overlapped between D-WF and D-FF. In January 2015, while $SUVA_{410}$ in D-FF remained relatively stable, it increased clearly in D-WF, similar changing pattern to that of $SUVA_{254}$.

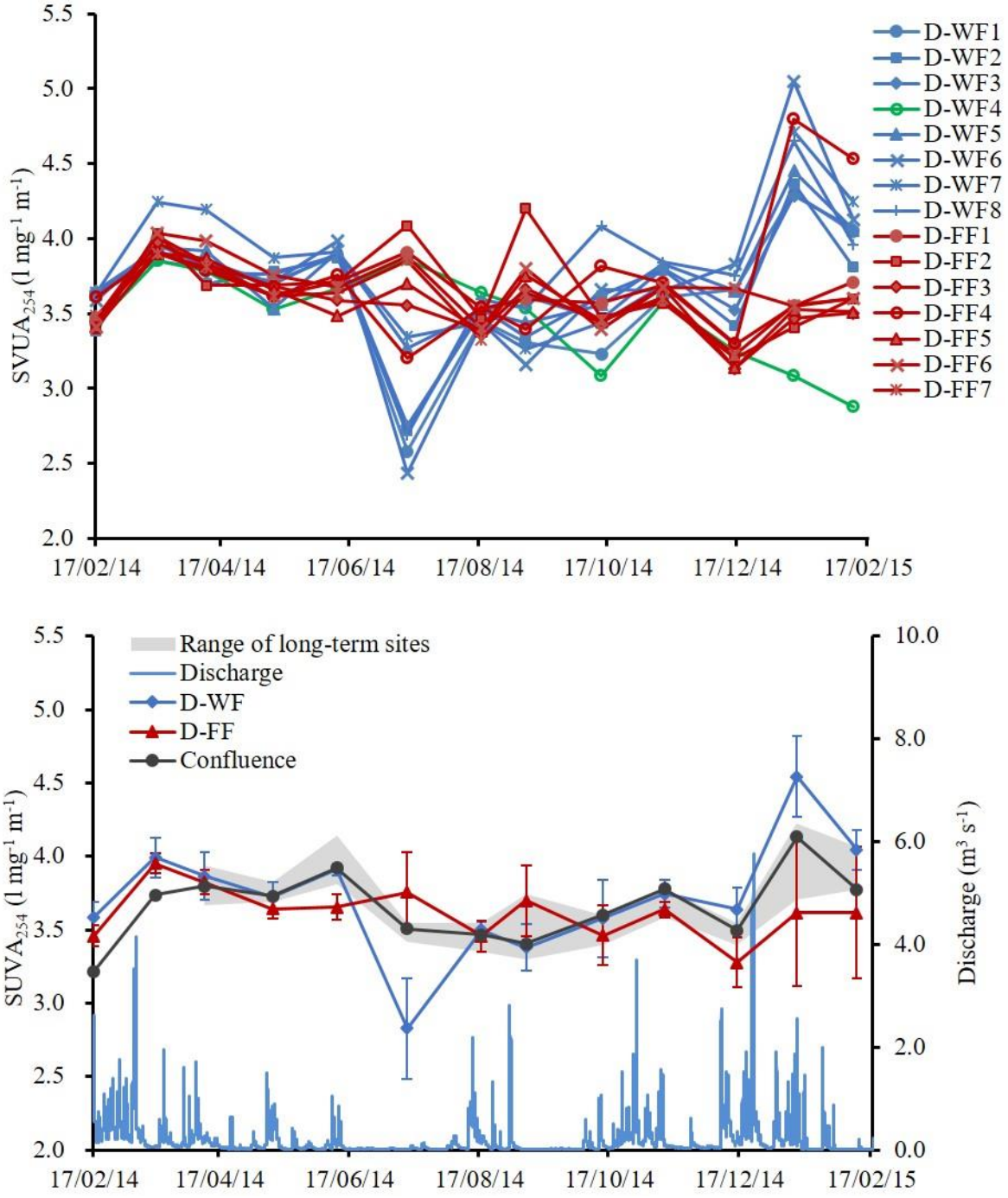


Fig. 4-9. $SUVA_{254}$ values of all sampling sites along the Drumtee tributaries (Fig. 4-1) for each month from February 2014 to February 2015 are shown in the upper panel. The mean values of each tributary and the confluence water are shown in the lower panel, where each sample point is the mean ± SD of all intra-stream points sampled on the same day. D-WF4 was pooled with D-FF. The grey area shows the range of $SUVA_{254}$ in the five Whitelee catchments.

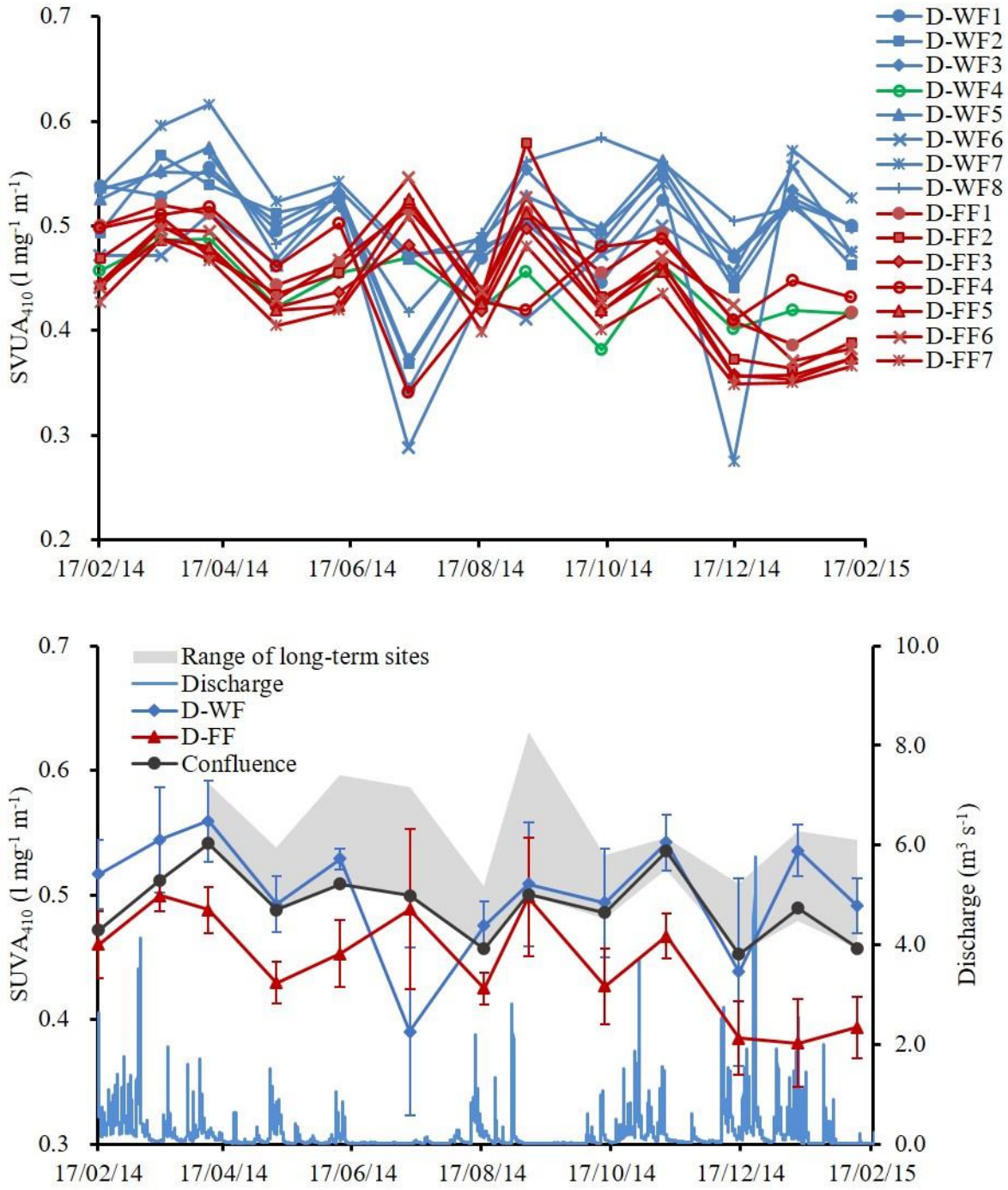


Fig. 4-10. $SUVA_{410}$ values of all sampling sites along the Drumtee tributaries (Fig. 4-1) for each month from February 2014 to February 2015 are shown in the upper panel. The mean values of each tributary and the confluence water are shown in the lower panel, where each sample point is the mean ± SD of all intra-stream points sampled on the same day. D-WF4 was pooled with D-FF. The grey area shows the range of $SUVA_{410}$ in the five Whitelee catchments.

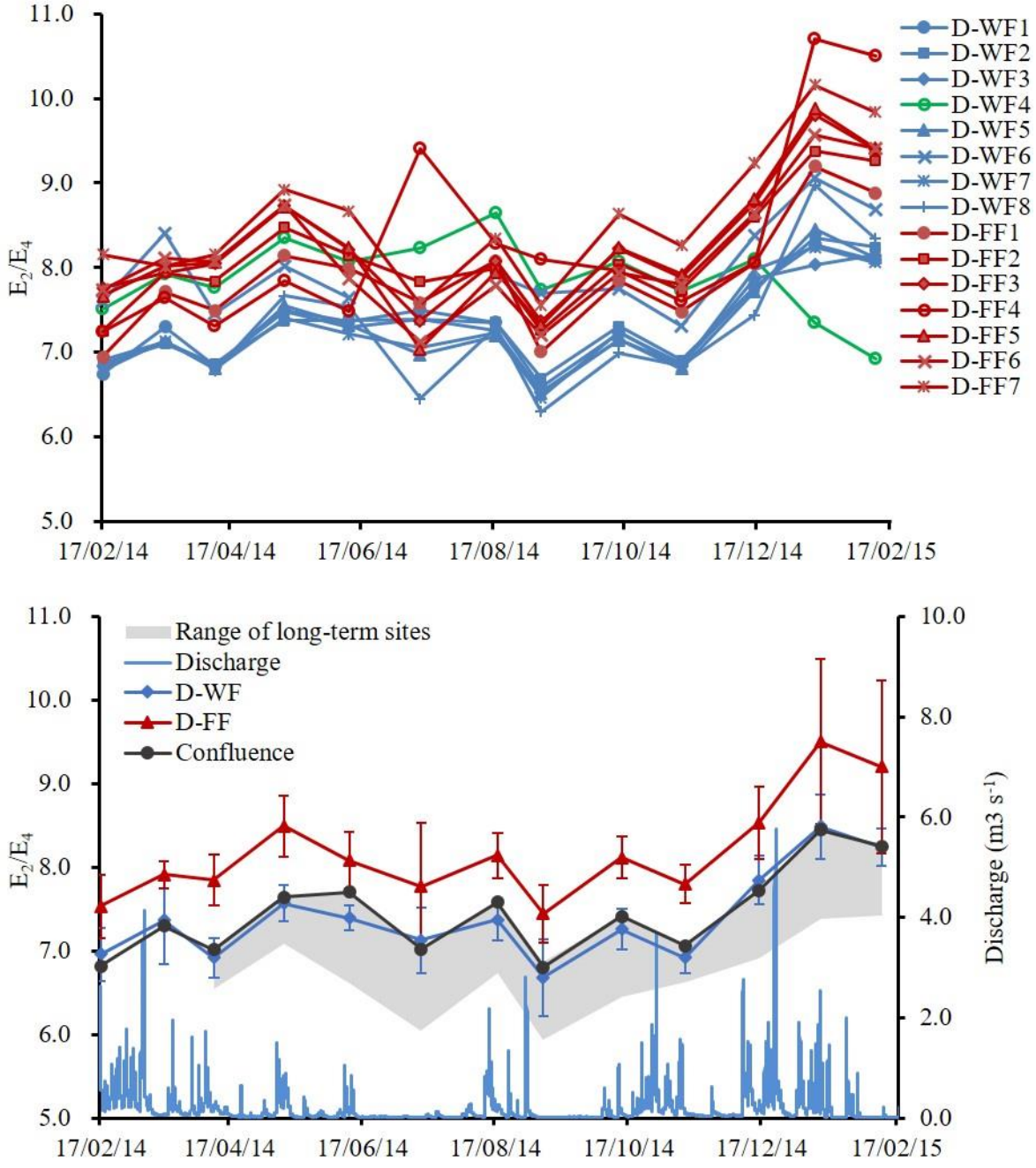


Fig. 4-11. E_2/E_4 values of all sampling sites along the Drumtee tributaries (Fig. 4-1) for each month from February 2014 to February 2015 are shown in the upper panel. The mean values of each tributary and the confluence water are shown in the lower panel, where each sample point is the mean ± SD of all intra-stream points sampled on the same day. D-WF4 was pooled with D-FF. The grey area shows the range of E_2/E_4 in the five Whitelee catchments.

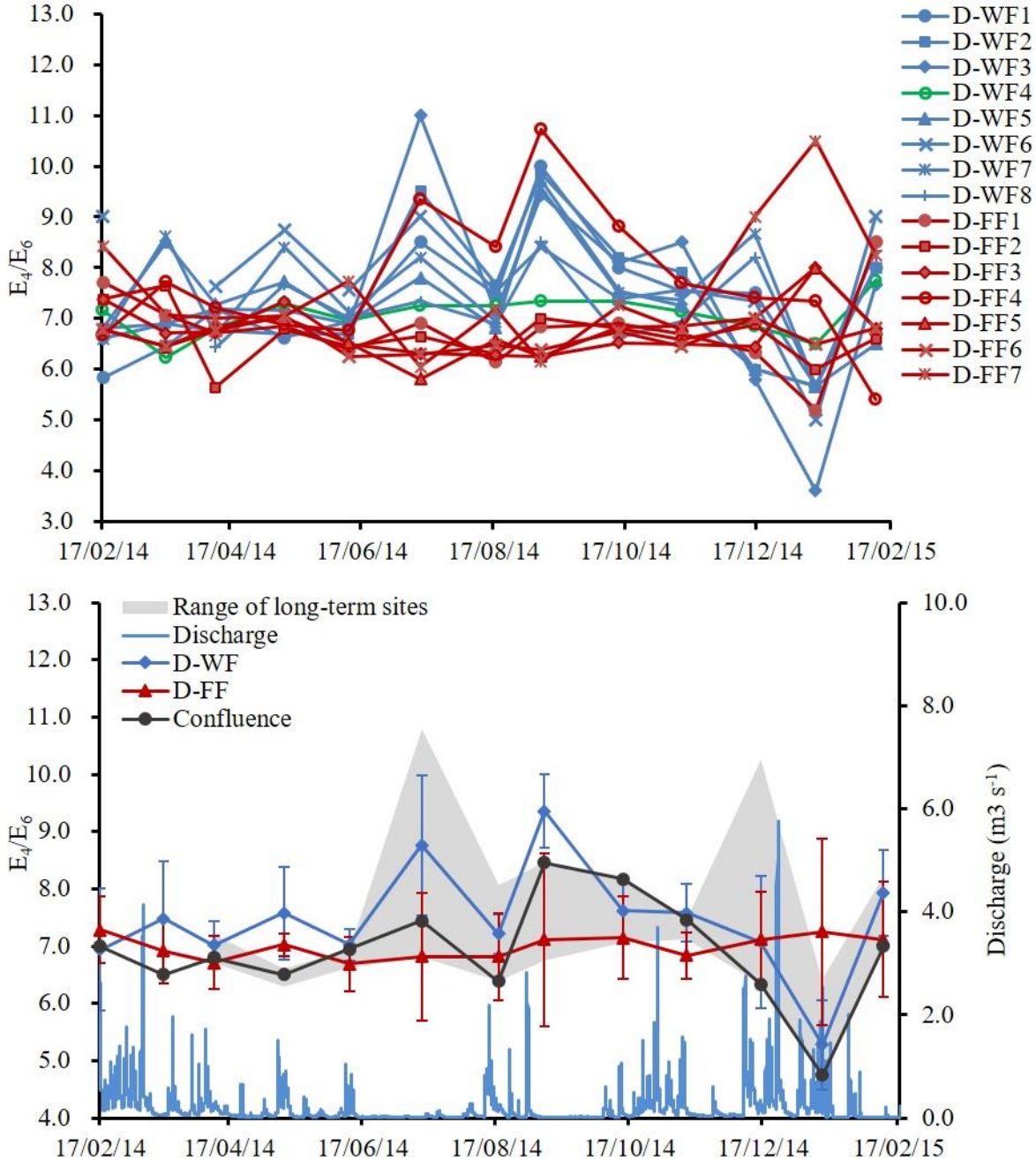


Fig. 4-12. E_4/E_6 values of all sampling sites along the Drumtee tributaries (Fig. 4-1) for each month from February 2014 to February 2015 are shown in the upper panel. The mean values of each tributary and the confluence water are shown in the lower panel, where each sample point is the mean ± SD of all intra-stream points sampled on the same day. D-WF4 was pooled with D-FF. The grey area shows the range of E_4/E_6 in the five Whitelee catchments.

Monthly E_2/E_4 ranged from 6.7 ± 0.5 – 9.5 ± 1.0 in Drumtee with an annual average of 7.4 ± 0.5 in D-WF and 8.2 ± 0.6 in D-FF (Fig. 4-11, Table 4-5). Both mean values were bracketed by the range of E_2/E_4 for terrestrial DOM (Table 4-5), indicating HS in Drumtee water were mainly derived from terrestrial sources. D-FF E_2/E_4 was larger than those in long-term sites, whilst D-WF was at the higher end of the range (Fig. 4-11). This suggests DOC in Drumtee may contain proportionally less HS compared to long-term sites. Within the Drumtee Catchment, the significant and constantly larger E_2/E_4 in D-FF ($p < 0.05$, Table 4-5) reveals the D-FF DOC had a smaller proportion of HS in aromatic C pool. E_2/E_4 in the confluence water overlapped with D-WF at most of the time. Seasonally E_2/E_4 in both streams showed similar and constant patterns from Feb. – Nov. 2014, which increased from November to January, when it was cold and with more intensive rainfall. This increase was also widely observed in the Whitelee long-term sites (Fig. 4-11).

A larger annual mean E_4/E_6 was measured in D-WF (7.4 ± 1.0) than that in D-FF (7.0 ± 0.2) ($p > 0.05$) over the year (Table 4-5). The ratios were not significantly different between the streams, indicating at most time, FA:HA was similar in the HS pools. Both E_4/E_6 generally fitted with the range in long-term sites and those found in other research as well (Fig. 4-12, Table 4-5). Drumtee E_4/E_6 was closer to those for streams with more FAs, suggesting HS pool was dominant with less humified materials. FA:HA was more variable in D-WF over the year, with the smallest as 5.3 ± 0.8 in January 2015, and the largest as 9.4 ± 0.6 in September 2014, whilst it remained constant in D-FF, ranging from 6.7 ± 0.5 – 7.3 ± 0.6 (Fig. 4-12). The changes in E_4/E_6 of D-WF also seemed to be more sensitive to hydrological conditions, as the ratio increased clearly in the dry months (July and September), and decreased in the wet winter month (January 2015).

The model in Fig. 4-13 illustrates the difference of DOC composition between Stream D-WF and D-FF. Due to the greater proportion of HS, but similar FA:HA in HS pool, the DOC in D-WF was more humified than D-FF.

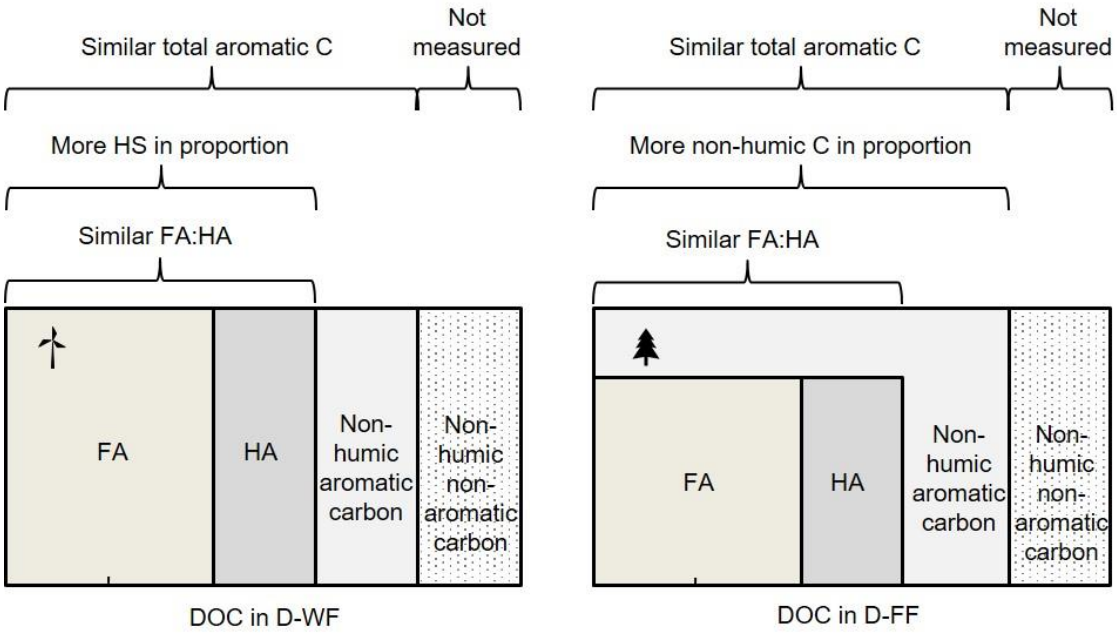


Fig. 4-13. Schematics showing the different proportions of HS in DOC produced from the two streams with the relative areas hypothesised to represent.

Table 4-5 A summary of the application of the spectrophotometric characterization used in this study, interpretation of each parameter, field ranges in other research and the key findings of two Drumtee streams comparison. ANOVA was applied for testing the significance of variance between two streams and it shows the mean values of $SUVA_{254}$ and E_4/E_6 were not statistically different between D-WF and D-FF with a $p > 0.05$.

Measurements	Other studied DOM	Field ranges	Authors	Drumtee
$SUVA_{254}$ ($lmg^{-1}m^{-1}$) Positively related to DOC aromaticity	OM isolates from ocean to dark water	0.6 – 5.3	(Weishaar et al., 2003)	D-WF: 3.7 ± 0.4 D-FF: 3.6 ± 0.2 ($p > 0.05$) Similar aromaticity
	Aquatic isolated FAs	0.6 – 3.9		
	Aquatic isolated HAs	5.1 – 5.3		
	New Zealand peat pore water	1.9 – 3.5	(Moore and Clarkson, 2007)	
	Yukon river	2.2 – 3.6	(Wickland et al., 2012)	
	$SUVA_{285}$ deforested peatland pore water	3.5 – 6.1	(Gandois et al. 2013)	
	Peatland well water	3.65 ± 0.04	(Olefeldt et al., 2013)	
	Peatland ditch water	4.58 ± 0.89		
	Pore water from blanket bog	4.00 ± 0.47	(Peacock et al., 2014)	
	Bog lake	4.58 ± 1.16	(Selberg et al., 2011)	
	Stream water from a peatland headwater	4.6	(Goulsbra et al., 2016)	
$SUVA_{410}$ ($lmg^{-1}m^{-1}$) Positively related to more complex aromatic C	Discharge from surface soil in peat	~0.35	(Lou et al., 2014)	D-WF: 0.50 ± 0.05 D-FF: 0.45 ± 0.04 ($p < 0.05$) D-WF DOC contains more complex aromatic C
	Discharge from -10 cm water depth in peat	~0.39		
	Discharge from -20 cm water depth in peat	~0.5		
	Streams and drains before peat drain-blocking	0.54	(Worrall et al., 2007)	
	Streams and drains after peat drain-blocking	0.67		
E_2/E_4 Inversely related to HS proportion	Terrestrial DOM	4 – 11	(Selberg et al., 2011)	D-WF: 7.4 ± 0.5 D-FF: 8.2 ± 0.6 ($p < 0.05$) D-WF DOC contains more HS than D-FF
	Ditch water from blanket bog	6.42 ± 0.73	(Peacock et al., 2014)	
	Pore water from blanket bog	6.77 ± 0.45		
	Downstream water from a peaty catchment	15.5	(Graham et al., 2012)	
E_4/E_6 Positively related	HAs extracted from soils	≤ 5	(Kononova, 1966)	D-WF: 7.4 ± 1.0 D-FF: 7.0 ± 0.2
	FAs extracted from soils	6.0 – 8.5		

to FA:HA	HAs extracted from soils	2 – 5	(Thurman, 1985)	($p > 0.05$)
	FAs extracted from soils	8 – 10		
	Blanket peat headwater in England	2.8 – 7.3	(Worrall et al., 2002)	Similar HA:FA between streams
	Blanket peat headwater in England excluding storm events	~5 – 6		
	Stream water from a peatland headwater	6.5	(Goulsbra et al., 2016)	
	Ditch water from blanket bog	5.99 ± 3.76	(Peacock et al., 2014)	
	Pore water from blanket bog	7.37 ± 4.04		

4.4.6 Stream [SRP] and [TON] time series (2006 – 2016) in the Whitelee catchments

During the last ten years, [SRP] in the south of the Whitelee wind farm ranged from 1.0 – 164.3 μg l^{-1} (noting that extreme high values could represent contamination from a point source), with the largest at WL13 in August 2008 and the smallest at WL15 in March 2007 (Fig. 4-14).

The time series of [SRP] showed clear increases at WL13 and 14 during the original wind farm development. WL13 had the greatest increase in the first phase of development, with the median [SRP] increasing from 17.3 μg l^{-1} in HY2006 - 07 to 75.2 μg l^{-1} in HY2007 – 08 (Fig. 4-15). The median value started to reduce from HY2008 – 09 (72.5 μg l^{-1}), the later period of the original development. The similar temporal changing pattern was observed in WL14 median [SRP] over the ten years, which largely increased from 16.0 to 62.1 μg l^{-1} in the early development period and decreased afterwards. However the median [SRP] at both sites were still greater than those in the first year (HY2006 – 07) (Fig. 4-15).

During July 2006 – October 2010, median [SRP] at WL13 and 14 was greater than 40 μg l^{-1} (the limit of [SRP] less than which water quality is 'good'), while the concentrations at other sites ranged from 20 – 30 μg l^{-1} (Table 4-6).

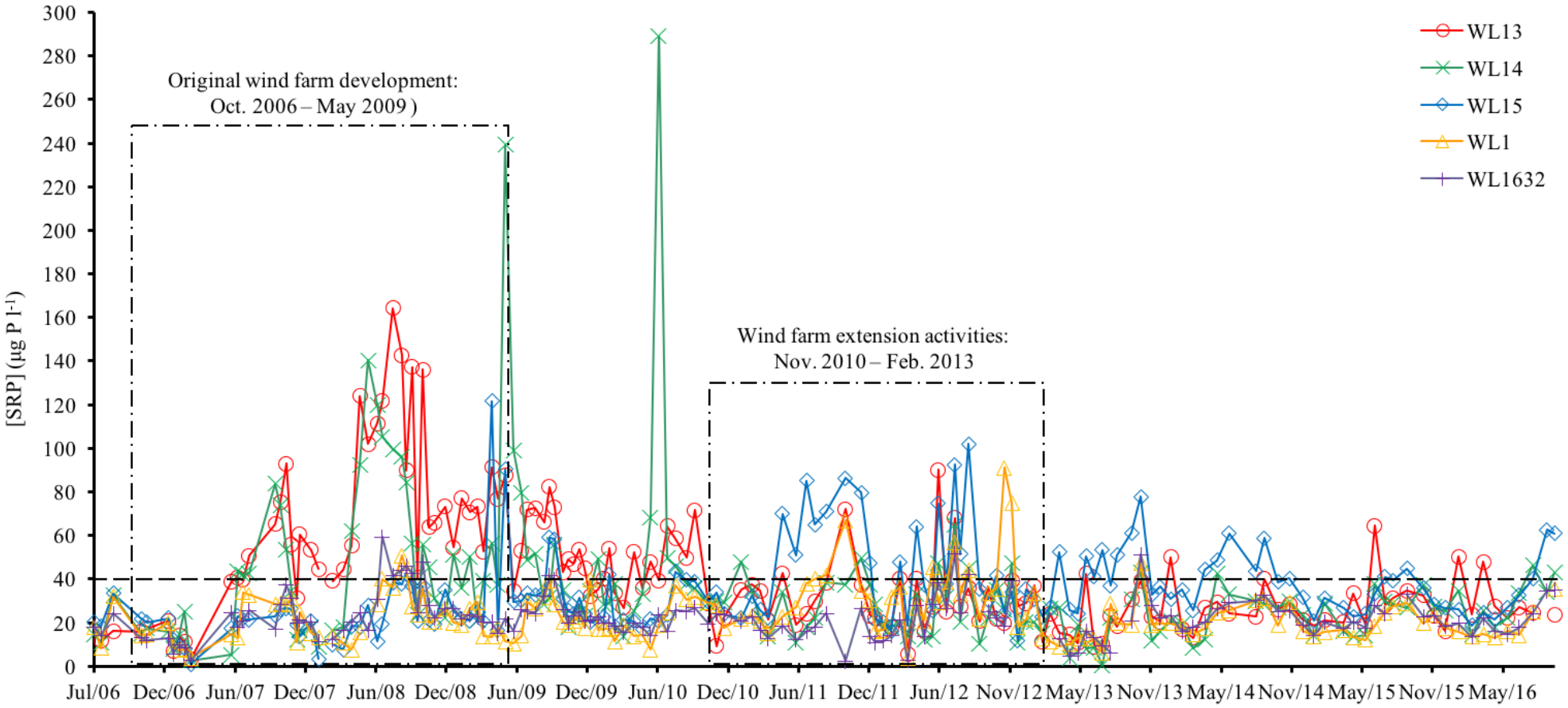


Fig. 4-14. Long-term time series (July 2006 – Sept. 2016) of [SRP] for catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). The black dashed line is the limit [SRP] less than which water quality is 'good'. Dash boxes indicate time periods of original windfarm development (all catchments affected, Oct. 2006 – May 2009), and extension (WL15 affected most, Nov. 2010 – Feb. 2013). Data prior to Aug. 2011 was collected by Murray (2012) and Waldron et al. (2009), and between Sept. 2011 – Mar. 2013 by Phin (2015).

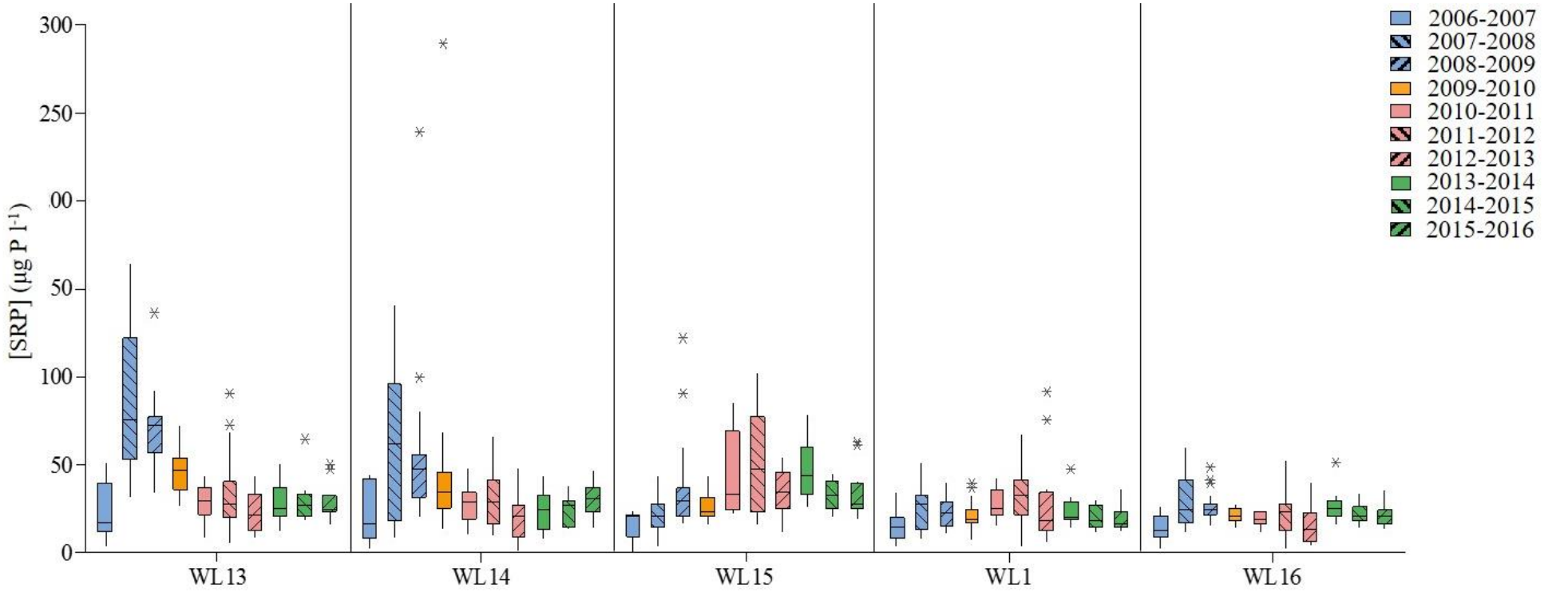


Fig. 4-15. Box-plots showing long-term time series (Oct. 2006 – Sept. 2016) of [SRP] for catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Each coloured bin is pooled data for a hydrological year. The boxed area shows interquartile range, the median is the horizontal line within the box, and the vertical lines extending from the boxes show the minimum and maximum. Data point outliers are shown as asterisks. Data prior to Aug. 2011 was collected by Murray (2012) and Waldron et al. (2009), and between Sept. 2011 – Mar. 2013 by Phin (2015).

Table 4-6 Median values, p values, significance and trend slope for [SRP] in catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Data was grouped into three: the total 10 years, July 2006 – Oct. 2010 for the first wind farm development and one year afterwards, and Nov. 2010 – Sept. 2016 for the extension and three years afterwards. Numbers in bold red are indicating the largest trend slopes for significant changes. * p < 0.05, ** p < 0.01, *** p < 0.001.

Years	Sites	Median [SRP] (µg l^{-1})	p	Significance	Slope
July 2006 – Sept. 2016	WL13	42.84	0.000	***	**-3.73**
	WL14	36.48	0.081		-0.95
	WL15	34.24	0.000	***	1.41
	WL16	22.31	0.352		0.27
	WL1	23.66	0.802		0.09
July 2006 – Oct. 2010	WL13	58.62	0.694		-3.65
	WL14	48.22	0.325		2.90
	WL15	26.89	0.000	***	**2.57**
	WL16	23.23	0.431		0.40
	WL1	21.81	0.168		1.60
Nov. 2010 – Sept. 2016	WL13	28.64	0.811		0.20
	WL14	25.76	0.953		0.09
	WL15	40.85	0.003	**	**-2.35**
	WL16	21.39	0.583		0.29
	WL1	25.33	0.004	**	-2.06

Further during the extension activities, a clear increase in [SRP] was observed at WL15, while it remained relatively constant or increased slightly at other sites (Fig. 4-14). The maximum and median [SRP] of WL15 increased rapidly from HY2009 – 10 to HY2011 - 12 (maximum: from 43.3 to 102.0 µg l^{-1}; median: from 23.5 to 47.5 µg l^{-1}, the first two year of extension activities. The high [SRP] at WL15 was maintained afterwards although a decrease was observed ($p < 0.01$, Table 4-6, Fig. 4-15). During Nov. 2010 – Sept. 2016, WL15 was the only site having median [SRP] greater than 40 µg l^{-1} (Table 4-6), while it was stable and smaller at other sites (except WL1).

Overall, there was a significant decrease of [SRP] at WL13 after the observed increase in 2008, and a significant increase at WL15 during the ten years (Table 4-6).

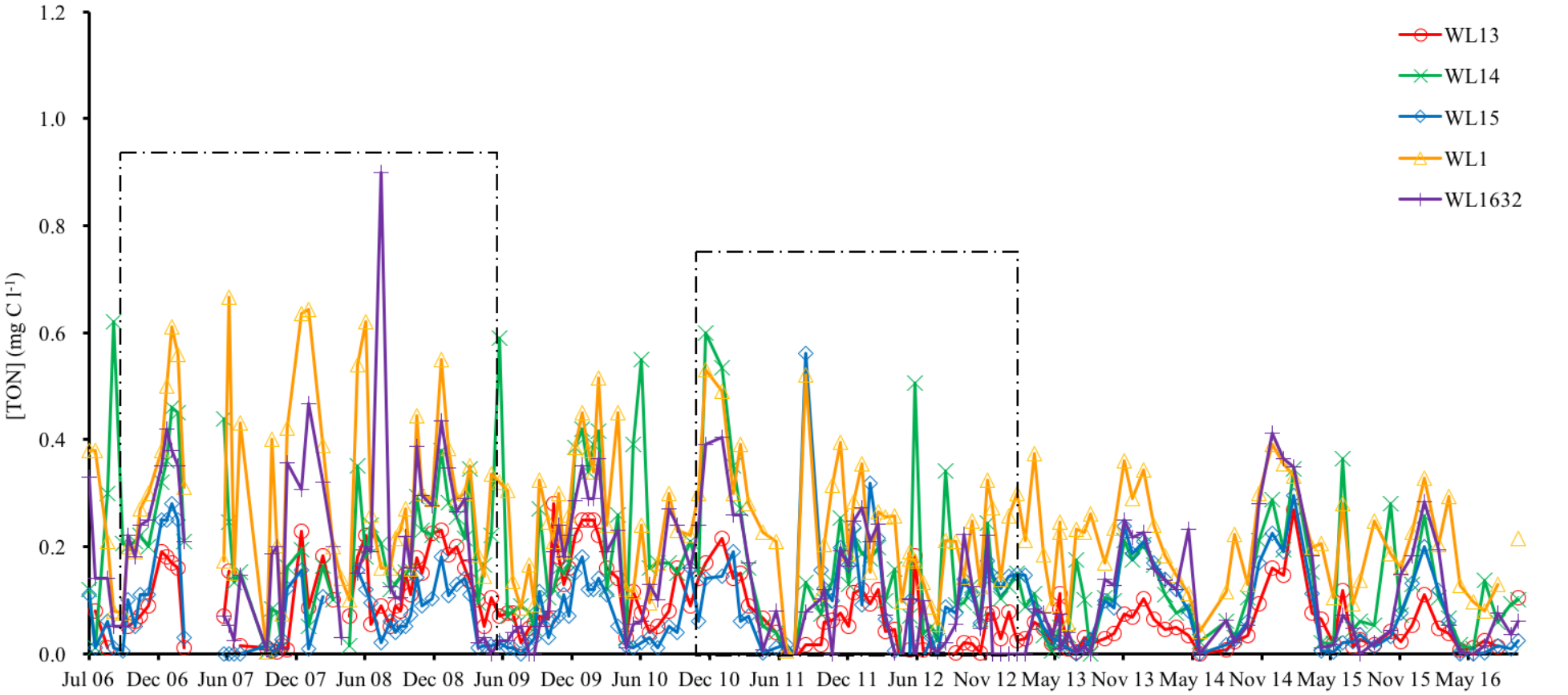


Fig. 4-16. Long-term time series (July 2006 – Sept. 2016) of [TON] for catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Dash boxes indicate time periods of original windfarm development (all catchments affected, Oct. 2006 – May 2009), and extension (WL15 affected most, Nov. 2010 – Feb. 2013). Data prior to Aug. 2011 was collected by Murray (2012) and Waldron et al. (2009), and between Sept. 2011 – Mar. 2013 by Phin (2015).

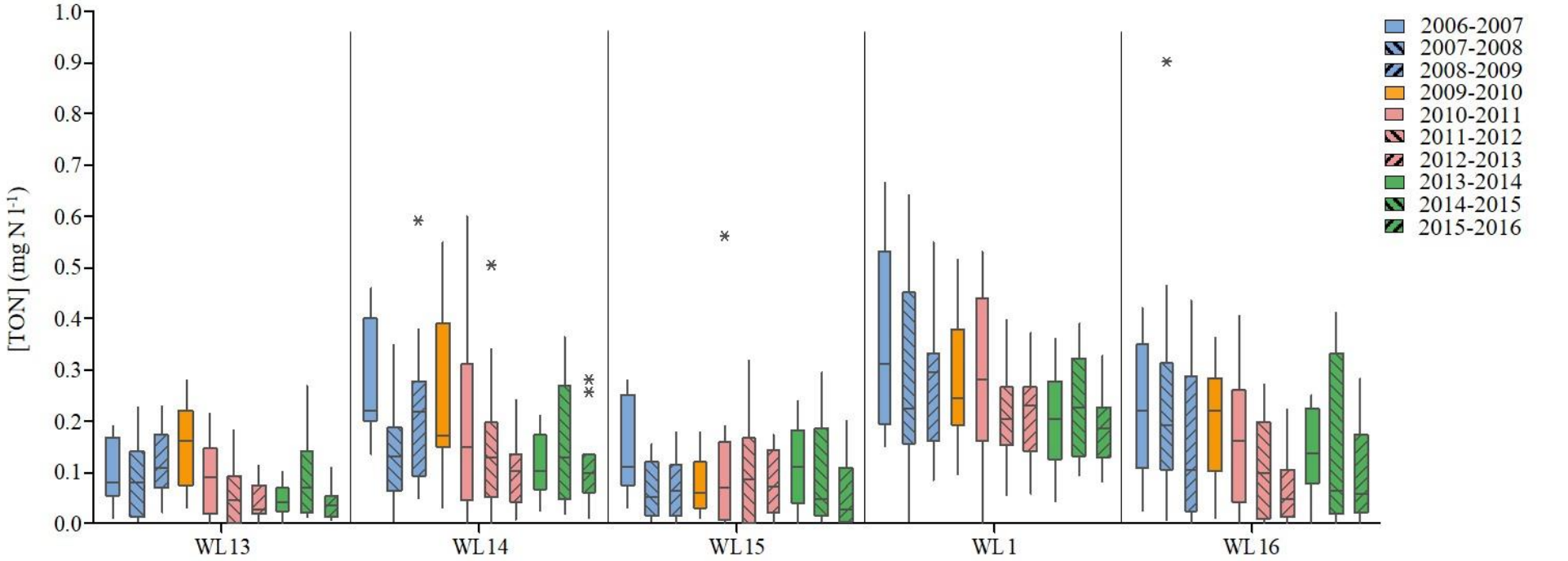


Fig. 4-17. Box-plots showing long-term time series (Oct. 2006 – Sept. 2016) of [TON] for catchments WL13, 14, 15, 16, 1 draining the south of Whitelee wind farm (Fig. 2-1). Each coloured bin is pooled data for a hydrological year. The boxed area shows interquartile range, the median is the horizontal line within the box, and the vertical lines extending from the boxes show the minimum and maximum. Data point outliers are shown as asterisks. Data prior to August 2011 is from (Murray, 2012; Waldron et al., 2009), between September 2011 – March 2013 from (Phin, 2015).

Table 4-7 Median values, p values, significance and trend slope for [TON] in catchments WL13, 14, 15, 16, 1 draining the south of the Whitelee wind farm (Fig. 2-1). Data was grouped into three: the total 10 years, July 2006 – Oct. 2010 for the first wind farm development and one year afterwards, and Nov. 2010 – Sept. 2016 for the extension and three years afterwards. Numbers in bold red are indicating the largest trend slopes for significant changes. * p < 0.05, ** p < 0.01, *** p < 0.001.

Years	Sites	Median [TON] (mg l^{-1})	p	Significance	Slope
July 2006 – Sept. 2016	WL13	0.07	0.000	***	**-0.01**
	WL14	0.15	0.000	***	**-0.01**
	WL15	0.08	0.241		0.00
	WL16	0.15	0.000	***	**-0.01**
	WL1	0.24	0.001	***	**-0.01**
July 2006 – Oct. 2010	WL13	0.10	0.166		0.01
	WL14	0.20	0.849		0.00
	WL15	0.07	0.318		-0.01
	WL16	0.19	0.637		-0.01
	WL1	0.27	1.000		0.00
Nov. 2010 – Sept. 2016	WL13	0.05	1.000		0.00
	WL14	0.11	0.480		-0.01
	WL15	0.09	0.211		0.00
	WL16	0.10	0.127		-0.01
	WL1	0.23	0.155		-0.01

[TON] in the Whitelee catchments ranged from 0 – 0.90 mg l^{-1} from Jul. 2006 – Sept. 2016, with the greatest in WL16 in August 2008, and [TON] below detection limit in some of the streams, often in summer (Fig. 4-16). [TON] appears to have a seasonal trend with usually highest concentrations in the winter and lowest concentrations in the in summer. Generally, there were greater [TON] in WL1 than other sites throughout the ten years (Fig. 4-16 and Fig. 4-17), with the WL1 median [TON] of 0.24 mg l^{-1} and ≤ 0.15 mg l^{-1} in the other catchments (Table 4-7).

During the ten years since wind farm commenced, there were small but significant decreases in all long-term sites except for WL15, which showed an increase of the maximum [TON] in 2011 (few months after the extension started) (Fig. 4-17, Table 4-7). However, there were no significant changes observed either during the first wind farm development and two years afterwards (2006 – 2010), or the following six years since the extension activities commenced (2010 – 2016).

4.5 Discussion

4.5.1 Long-term monitoring: influence of wind farm disturbance on [DOC]

The seasonal pattern of changing [DOC] is consistent with that observed in Whitelee in previous research (Waldron et al. 2009; Murray 2012; Phin 2015) and in other temperate peatland systems (e.g. Worrall et al. 2006; Dawson et al. 2011), with the greatest [DOC] in late summer and the smallest in winter and spring. The concentration maximum in summer is likely caused by most terrestrial productivity and lower water table promoting DOC production (Freeman et al., 2001), with pore water DOC flushed into streams when the catchment wets up (Tipping et al., 2007). The [DOC] range across Whitelee (including long- and short-term sites) was large from 5.2 – 89.3 mg l^{-1}, which is comparable to other peatland catchments in UK but the Whitelee catchments are at the higher end of this range. For example, peatland sites across Scotland were observed to have [DOC] up to 88 mg l^{-1} (Billett et al., 2006; Dawson et al., 2011, 2004; Grieve and Gilvear, 2008), and in England and Wales up to 60 mg l^{-1} (Goulsbra et al., 2016; Jones et al., 2016; Moody and Worrall, 2016; Pawson et al., 2012; Worrall et al., 2007).

Median [DOC] and seasonal trend analysis showed there were significant increases in all long-term sites across last ten years. The last time when part of this data set was considered, it was suggested that [DOC] recovered to its original concentration after five years following development (Phin 2015). However, in this research, with a longer observation period, [DOC] was found to decrease during HY2010 – 11 and HY2012 – 13 in some of the sites, but in general to show an increasing trend since wind farm construction started in 2006 (Fig. 4-3). In WL15 there was no decreasing trend of [DOC] four years after the extension finished. Thus the decrease observed by Phin (2015) could be an annual variation of [DOC] with time. However, there was no control site in this long-term monitoring programme, and no other [DOC] data from comparable systems is available from locations close to Whitelee. Therefore, it cannot be assessed fully whether wind farm development is the controlling factor on the long-term [DOC] increase in Whitelee, as the effect of a climatic regional driver can be ascertained. Thus, the parsimonious interpretation here is that, as the most intensive land use change in Whitelee during the past ten years, wind farm development may exert local impacts on the observed [DOC] increase.

The wind farm development may also cause pulse of [DOC] in the draining waters. At WL13 and WL15, which were the catchments most affected by the original and extension

to wind farm development, an increase in [DOC] is observed which is consistent with disturbance from wind farm construction. During the period of original development WL13 [DOC] increased rapidly, and showed the greatest [DOC] of all catchments during construction and for two years after construction finished. However, when extension activities were commissioned, the greatest [DOC] occurred in WL15 and was maintained for subsequent years. Disturbance direct link between increased [DOC] as a result of wind farm construction is difficult as there is not a tracer. However, for two sites to respond to construction in the catchment with increased [DOC], independent of other sites that would have experienced similar climatic conditions and so rainfall and likely discharge, is strong supporting evidence that the construction is a more important driver of this [DOC] increase.

It is likely that the increasing [DOC] is due to wind farm-related peat disturbance (as the other macronutrients also evidence this, see section 4.5.2), but other drivers unrelated to the windfarm may also be important, for example, [DOC] may simply increase as less rainfall to dilute the soil water [DOC]. Indeed, there is a weak trend for decreasing discharge during HY2011 – 16 (Fig. 4-4). Further, the lack of continued increase in DOC export year-on-year shows factors other than land use change control within-stream loading. The export load will depend on i) the availability of pre-formed [DOC] in the porewater; ii) within season production rates of [DOC] and iii) flushing of the system (i.e. how wet it is, which will also influence water table depth and so [DOC] production. Additionally, the concentration measured in the fluvial system, and so the export calculated, will reflect net DOC processing within the stream e.g. in low flow, residence time is longer and there is more capacity for DOC removal by in-stream processes (Tranvik and Jansson, 2002). These mixed responses of [DOC] to discharge suggest other factors may exert more controls on the observed [DOC] increase. It is very difficult to unpick the controls on this without some additional field-based experiments, for example using soil lysimeters to sample and monitor porewater [DOC] over time and under different flow regimes to assess the interaction of production and export (e.g. Camino-Serrano et al., 2016).

The one-year detailed survey

There are mainly two aspects of wind farm-related peat disturbance: deforestation and construction activities (e.g. building turbines and access tracks). In one of the Whitelee catchments, Drumtee, the influence of these two different land uses on exported DOC was further revealed. Stream D-FF draining the felled forestry had constant greater [DOC] during the one-year survey, while the smaller [DOC] in D-WF was in the lower end of

Whitelee [DOC] range. The two streams drain from mainly dystrophic blanket peat with similar peat coverage (around 70 – 80%), thus it is maybe not likely that the [DOC] difference was a major result of soil difference. Both land use changes (turbines emplacement vs. felled forestry) cause disturbance to soil (Mitchell et al., 2010; Schelker et al., 2014; Zerva and Mencuccini, 2005). Both peat excavation in the turbine-covered area and tree felling in the felled area can leave an exposed surface more susceptible to C losses (Glatzel et al., 2003). However, clear-felling and mulching results leave large residues of tree litter and dying tree roots, which are easily decomposed (Zerva and Mencuccini, 2005). Meanwhile the removal of forest canopy can increase soil and surface temperatures (Hoffmann et al., 2003; Jauhiainen et al., 2012), and the decomposition of branches and litter often releases nutrients to the soils (Kreutzweiser et al., 2008), both of which can enhance peat and wood litter decomposition (Gandois et al., 2013). Thus, in this dystrophic blanket peat dominated area, these conditions very likely stimulate microbial activity, thereby altering rates of peat decomposition and generation of new DOC from felled material. Therefore, it is likely that the high [DOC] in D-FF is a result of historical drainage for commercial forestry and now residue breakdown from deforestation. This result further supports the previous suggestion that deforestation can have greater influence on [DOC] than the excavation disturbance associated with the wind farm turbine, cabling and road emplacement.

In previous research about wind farm and C cycling, deforestation has been suggested to be most likely control on carbon concentration and/or export (Murray 2012; Phin 2015; Smith 2016). With two different landscapes (turbines emplacement vs. felled forestry) in one small catchment Drumtee, how different components of wind farm construction causing different land use changes may influence exported DOC concentration has been now further examined and more clearly demonstrated in this research.

4.5.2 Long-term monitoring: influence of wind farm disturbance on [SRP] and [TON]

Phosphorus is an important element for building biomass and normally is presented in surface water in low concentrations (McMahon and Read, 2013). In most Whitelee long-term sites, [SRP] did not show a significant increasing/decreasing change across the ten years. Instead, in WL13 and 14, increases were observed following the start of original wind farm development (during 2007 – 2008, Fig. 4-15). A similar [SRP] increase occurred again in WL15 after the extension development started. According to standards for [SRP] by the Water Framework Directive (2000) UK Technical Advisory Group, which defines water quality as ‘good’ when [SRP] < 40 ug l^{-1}, a negative impact on WL13 and 14 stream water quality was apparent in the period after wind farm construction started.

Runoff from surfaces of forestry, cultivated lands and pasture (Withers and Jarvie, 2008) can be one of the major P sources entering streams, and forest harvesting has been suggested to increase nutrient concentrations in adjacent streams (Feller, 2005). WL13 and 14 experienced 30.3 % and 10.3 % deforestation due to the original wind farm development and were closer to the original felled area than the other sites, and WL15 hosted most of the extension construction and deforestation, which makes the [SRP] pulse in these catchments less surprising. [SRP] was also higher in WL15 upper catchment drainage hosting felled areas (Phin 2015). The timescale of [SRP] recovery to baseline levels described in this project is similar to the response to deforestation observed in other peatland catchments (Cummins and Farrell, 2003a, 2003b; Rodgers et al., 2010), and the subsequent four-year trend of [SRP] no longer increasing after the extension development further supports the interpretation the increase was a response to the impacts of tree-felling.

Since a large area of deforestation occurred in Whitelee, an increase of [TON] may have been expected, as it has been reported in other catchments subjected to extensive clear-felling (Neal et al., 2003; Tetzlaff et al., 2007). The export of N has normally been analysed in the context of forest management, and leftover leaves and branch are suggested to be the main source for N (Kaila et al., 2012). An increase was recognized in WL13 and WL14 during the original development and one year afterwards (2007 – 2010), but was not so in WL15 during the extension period (Fig. 4-17). Further no significant increase of [TON] was observed in any period of development, and instead decreasing trends were observed for most of the long-term sites. This general decrease may be within the degree of TON natural variation in the Whitelee system. However, another possibility could be that the continuously released P from felled debris facilitated more microbial activity, which consumed larger amount of N than before deforestation.

Cycling of micronutrients, particularly carbon, nitrogen and phosphorus are known to be related via microbial activities and stoichiometric composition across various ecosystems (Coble et al., 2016; Schlesinger et al., 2011). Ecological stoichiometric theory (Sterner and Elser, 2002) suggests the decomposition of DOC into CO_2 is strongly related to availability of N and P. As a result, the potential may exist in the years of wind farm development that decomposition of DOC in streams could be influenced significantly when exported [TON] and/or [SRP] from the Whitelee catchments also changed (although there was no way to track back now). Additionally, the converse is true, with DOC composition, in addition to concentration, influencing the uptake of ammonium and SRP in forested streams (Coble et al., 2016).

4.5.3 Short-term observation of spatial variation of DOC composition in the Whitelee and Drumtee catchments

4.5.3.1 DOC is more resistant in Whitelee than many other aquatic systems

DOC from the Whitelee catchments had $SUVA_{254}$ (2.2 – 4.5 l mg^{-1} m^{-1}) in the range of other fluvial environments (0.6 – 5.3 for organic matter isolates ranged from ocean to dark water) and towards the higher end. Whitelee $SUVA_{254}$ was greater than FAs (2.9 – 3.9 for most aquatic isolated FAs) but smaller than HAs (5.1 – 5.3, Weishaar et al. 2003; Table 4-5). This suggests DOC in all Whitelee catchments was rich in complex heterogeneous aromatic OM (Selberg et al., 2011) and more humic than many of other aquatic DOC. The result was not surprising as all these five catchments are peat-dominated (62 % - 90 % of each catchment area was covered by peat-related soils, see Chapter 2 Section 2.1.1). The annual mean $SUVA_{254}$ across sites, was close to those of peatland well water (3.65 ± 0.04,Olefeldt et al., 2013; Table 4-5) or blanket bog pore water (4.00 ± 0.47, Peacock et al., 2014; Table 4-5), but less than that of water from peatland ditch (4.58 ± 0.89, Olefeldt et al., 2013; Table 4-5) or a bog lake (4.58 ± 1.16, Selberg et al., 2011; Table 4-5). This may suggest the source of DOC was largely derived from soil pore water and/or groundwater/baseflow. Peatland is an important carbon sink, and 'old' DOC would be expected in the peatland soil and stream waters (Kalbitz et al., 2003), particularly that derived from the deeper peat layer or groundwater (Tipping et al., 2010). Moreover, older and thus more resistant DOC was detected in disturbed systems than intact ones (e.g. Moore et al., 2013), which is consistent with that Whitelee was undergoing disturbance from wind farm development on peat.

No clear seasonal pattern was observed for the changes in DOC composition across the Whitelee catchments (Fig. 4-6 and Fig. 4-7). This lack of apparent seasonal pattern in DOC composition has been observed elsewhere in both soil and stream waters (Seifert et al., 2016). Spatially, $SUVA_{254}$ and E_4/E_6 varied little between the Whitelee catchments during 2014 – 2016 ($p > 0.05$), indicating that DOC aromaticity and HA:FA in HS pool were broadly similar across the catchments. This understanding is refined by the $SUVA_{410}$, which $SUVA_{410}$ reflects the amount of complex aromatic carbon (mainly HS consisting of HAs and FAs, which are produced from humification process). Significantly smaller $SUVA_{410}$ was measured in the DOC of Drumtee confluence water (WL13) compared to the other Whitelee catchments, except for WL14 ($p < 0.05$, Table 4-4), suggesting in the Drumtee Catchment less DOC was formed of complex aromatic materials. The larger E_2/E_4 composition in Drumtee is consistent with less DOC humification and thus a smaller proportion of HS (HAs and FAs) (Kalbitz et al., 1999). As a result of proportionally less HS

but similar FA:HA within HS pool, DOC in Drumtee was less humic, and thus likely to be less resistant than other sites (Fig. 4-8).

4.5.3.2 Fewer nutrients and shorter water residence time may contribute to the less resistant DOC in Drumtee than other Whitelee catchments

There are both biotic (e.g. microbial activity) and abiotic factors (e.g. soil properties, temperature and hydrological regimes) which have impacts on the in-stream DOC dynamics and composition changes (Kellerman et al., 2014; Seifert et al., 2016). Among different environmental variables interacting with DOM, water residence time is one of the major controls shaping the molecular composition of DOM (Kellerman et al., 2014). The rate of organic carbon decay decreases with the increasing water retention time across different systems, leading to a decrease in organic carbon reactivity along the water continuum (Catalán et al., 2016), reflecting the more recalcitrant organic carbon pool. This may explain partially the less aromatic and humic DOC composition in Drumtee (WL13) than other Whitelee catchments. Drumtee had a much smaller catchment area (5.7 km^2) than other catchments (11.3 – 31.1 km^2), and the shortest flow length from outlet sampling site upstream to the nearest disturbance (around 2.5 km, Fig. 2-1) compared to others. In Drumtee the water retention time before reaching to sampling site perhaps would be shorter, and thus a smaller chance of organic carbon decomposition, leading to the less compositional shift from labile to recalcitrant C pool (indicated by smaller $SUVA_{410}$ in WL13, Table 4-4).

Furthermore, nutrient, especially nitrate concentration has been reported to influence organic matter composition, and is positively related to organic matter aromaticity (Seifert et al., 2016). Generally, nutrients including N and P can stimulate microbial DOC consumption, and the observed abundance of more aromatic compounds might be due to the depletion of low-molecular-weight compounds by active microbial degradation processes (Seifert et al., 2016). Here smaller [TON] in Drumtee (WL13) was measured during 2014 – 2016, while it was clearly larger in WL14, 16 and 1 (Fig. 4-16), but similar in [SRP] between sites. As N is important limiting element in the Whitelee catchments, the difference in N availability may closely change microbial activities and further act as another reason contributing to the less aromatic DOC in the Drumtee Catchment.

4.5.3.3 Wind farm disturbance can also affect DOC composition: example from Drumtee Catchment

Land use influences soil properties, such as pH, moisture, temperature and nutrient availability, and so has been suggested to affect DOC composition ultimately. From the comparison of Drumtee DOC optical properties between streams D-WF and D-FF, it is

possible to assess how the two specific wind farm-related land uses (turbines emplacement vs. felled forestry) affect DOC composition. As with Drumtee and the other Whitelee catchments, spatial variation in DOC composition also existed between tributaries D-WF and D-FF. The similar $SUVA_{254}$ and E_4/E_6 at most times of year, but smaller $SUVA_{410}$ and larger E_2/E_4 in D-FF, suggest less HS in D-FF, although a similar proportion of HAs. Thus DOC was more humic, and likely more resistant in D-WF than D-FF (Fig. 4-13).

Increased humification in D-WF is suggested by the constantly greater $SUVA_{410}$ (only apart from the dry months) and smaller E_2/E_4. The larger proportion of HS in D-WF suggests the DOC to be more humified. This difference can arise as either D-FF has inflow from a source of less humic material, or there is more HS delivery to D-WF. There are two mechanisms for this. Firstly, more non-HS were produced in D-FF catchment as a result of deforestation. Leachates from fresh litter are usually dominated by low-molecular-weight carbohydrates and easily decomposed by microbial (Marschner and Kalbitz, 2003), while highly aromatic high-molecular-weight (HMW) humic and fulvic acids have significant contribution to those from humified organic soils, such as peat (Kalbitz et al., 2003b; Olefeldt et al., 2013; Wickland et al., 2007). Young DOC consisting of more non-humic substances may be produced from the decomposition of tree litter in the felled area. There are no measurements of water table changes in this study, but due to the cessation of evapotranspiration, the depth to water tables beneath clear-felled stands is likely comparatively higher and the soil could have had a higher water content (Adams et al., 1991; Smethurst and Sadanandan Nambiar, 1995; Zerva and Mencuccini, 2005). Therefore, younger DOC with more non-humic substances from the upper soil layer may have been an important source of stream DOM. Secondly, the windfarm construction disturbance (mainly peat excavation and drainage) may have lowered the water table and exposed deeper DOC to an aerobic environment, more susceptible to DOC export when flushed during rainfall. The lowered water table can result in the reduction of phenolic compounds in response to oxygenation and thus trigger an 'enzymic-latch' mechanism, which accelerates further DOC humification, and produces 'older' and more resistant DOC, even after water table rose afterwards (Freeman et al., 2001; Worrall and Burt, 2005). It is difficult to tell which mechanism is more likely at this stage, or if both happened simultaneously.

4.5.4 Hydrological impacts on DOC composition in the Whitelee and Drumtee catchments

4.5.4.1 Hydrological impacts on DOC composition in the Whitelee catchments

While no consistent seasonal pattern in DOC composition was observed between either the Whitelee catchments or Drumtee tributaries, clear responses to climatic condition changes (e.g. hydrological condition) were seen in some of the months. The hydrological condition changes reflect modifications in flow paths, with subsequent DOC transportation from surface organic rich soil, via runoff or flow through soil macropores (Hinton et al., 1998; Vidon et al., 2008), and has been suggested to regulate DOM quality in headwater streams (Guarch-Ribot and Butturini, 2016).

Here, some 'sharp' increases or decreases in optical parameters of the Whitelee long-term sites were noticed when discharge changed. Generally in the Whitelee catchments, increased DOM humification was observed during the intensive high flow, e.g. January and November 2015, increased SUVA and decreased E_4/E_6 occurred across the catchments (Fig. 4-6 and Fig. 4-7 lower). Conversely DOC aromaticity and humification reduced during dry periods, for example, in the dry October in 2015 lower SUVA and higher E ratios were observed in all long-term sites (Fig. 4-6 and Fig. 4-7). These suggest exported DOC composition is sensitive to the changes of hydrological conditions.

Similar observations were recorded elsewhere (Clark et al., 2012; Fellman et al., 2009; Guarch-Ribot and Butturini, 2016; Nguyen et al., 2013). Hydrological conditions can exert indirect impacts on DOC dynamics and qualities via regulating microbial activities in soil, which increase when water table drops and contribute to more inputs of labile and less complex carbon compounds (Clark et al., 2012; Grand-Clement et al., 2014). Futhermore, the reduced solubility of the larger complex HS due to drought-induced acidification in soil water could also account for the observed decrease of DOC humification degree (Clark et al., 2012). Conversely when there are larger water flows, dissolution of more complex humic carbon could be favoured following decreases in acidity and solute strength. The observed increases in humic-like content, aromaticity, and humification degree during stormflows in this and other research (Fellman et al., 2009; Nguyen et al., 2013) may also be due to the progressively exhaustion of labile DOC stock available for export due to rainfall flush (Worrall, 2002). It should be noted, that there may be a contribution of authochthonous microbial production, normally less humic than terrestrial DOC, to the fluvial DOC pool, a process which can happen due to longer water residence time and potential fragmentation of river continuum (Vazquez et al., 2011).

4.5.4.2 Opposite responses of DOC composition to hydrological condition in Drumtee

The responses of DOC composition to hydrological change varied between the two tributaries in the Drumtee Catchment. The fluctuation of spectrophotometric characteristics of D-WF DOC and the consistency in D-FF shows that fluvial DOC in D-WF is more sensitive to rainfall and soil water depth. In July 2014, one of the dry months, D-WF DOC became less aromatic (decreased $SUVA_{254}$ and $SUVA_{410}$, Fig. 4-9 and Fig. 4-10) and had proportionally less HAs in HS (increased E_4/E_6, Fig. 4-12), which is consistent with the general observations in the Whitelee catchments (namely decreased DOC humification in the drier periods). However an increase in HS content was shown in D-FF DOC pool in July and September 2014 which were both dry months (Fig. 4-10 and Fig. 4-11).

Considering the similar soil types and climatic conditions within this small catchment, the different land uses may be linked to the opposite DOC composition changes when stream water flow was lower. As discussed earlier, the depth to water tables beneath the felled area can be comparatively higher than the directly exposed wind farm construction land as the tree mulch and lack of vegetation acted to reduce evaporation, and this may explain the less sensitivity of optical characteristics of D-FF to rainfall. In Drumtee Catchment, smaller stream water discharge implied less contribution from soil water, especially upper peat soils, therefore deeper soil water may contribute more to stream DOM. Deeper peat is more recalcitrant due to the residual accumulation of lignin, phenolic compounds and HS after labile carbon has been decomposed by microbial (Glatzel et al., 2006; Hogg et al., 1992). But meanwhile in a tropical deforested peatland, aromaticity of pore water DOC increased from the surface to 30 cm depth, then decreased to 180 cm, suggesting that aromatic molecules accumulated around 30 cm depth (Gandois et al., 2013). Based on the assumption that there could be less water table reduction in D-FF (the felled area), the increased DOC aromaticity in D-FF during dry months may be explained by the dominant DOC source from the more degraded deeper peat. Similarly more coloured and aromatic DOC in peatland soil water with the reduced water tables was also observed elsewhere (Lou et al., 2014). However in D-WF the water table might have dropped below the maximum accumulation of aromatic materials in the dry period. Drainage from mineral soil, groundwater and/or the production by microbial activities in soil and water may become dominant.

4.6 Conclusions

The purpose of this chapter was to:

1. assess if there are differences between catchments that have been subject to different amount of disturbance and are of different catchment scales

2. consider how land use changes (specifically wind turbine emplacement vs deforestation) have affected DOC concentration and composition by comparing within a site

3. consider from a 10 year time series if bulk DOC and nutrient concentrations have responded to an original windfarm construction and its extension.

From the long-term monitoring for water chemistry (July 2006 to September 2016), it is found in this wind farm-hosting peaty catchment, [DOC] and [SRP] increased during construction periods at the sites most affected by the wind farm development. A long-term increase was observed in [DOC] and this has not declined to original values after ten years. However, there was no control site during this 10-year monitoring, and no [DOC] available from the nearest comparable monitoring site to Whitelee to check background information. With noting this, a parsimonious interpretation may be that land use change by wind farm development was more responsible for the local [DOC] increase in Whitelee as a local factor. However, no increasing trend in DOC export was found in the Whitelee catchments, and so this must be influenced by other factors that control DOC delivery to the stream and fate within the stream. However, wind farm long-term disturbance may still result in increased DOC export by providing more available DOC at source, and increased DOC loss when discharge was large. Further, wind farm development may result in the inter-catchment differences in DOC export if there are difference in land development, as here WL15 exported more terrestrial DOC than WL13, but both were affected by the extension and original development respectively. The increasing impact on [SRP] was existing temporally for about one year. There was a small decreasing trend in [TON] across the Whitelee catchments, which may be due to the net N consumption, facilitated by the increased [SRP] after felling activities and/or increasing water residence time.

Generally, [DOC] in all sites showed similar range and seasonal pattern, with the maximum in summer/autumn and minimum in winter, reflecting the integration of catchment production and export. However, spatial differences were observed in Drumtee, namely the felled catchment had greater mean [DOC], 53.3 ± 24.4 mg l^{-1}, than the wind farm catchment, 27.1 ± 13.2 mg l^{-1}. This is attributed to differences in historic and recent land management. The soil disturbance e.g. peat excavation and track construction would release more DOC from peat to streams, but the greater [DOC] in D-

FF showed deforestation which resulted in the decomposition of mulched tree brunch and leave litters may have produced more DOC to export to streams than construction activities.

Spatial differences in DOC composition existed between WL13 and the other long-term sites, with DOC in WL13 being less humic and resistant, suggesting the significance of environmental factors including nutrient availability and water residence time. The larger [TON] and longer water retention time in other catchments could stimulate more active microbial activities and consumption of labile DOC pool, and hence more recalcitrant C would be retained for the down streams. The impact of land use on DOC composition was suggested by showing the difference between D-WF and D-FF in Drumtee. The constantly smaller $SUVA_{410}$ and greater E_2/E_4 in D-FF indicating 'fresher' DOC produced from the felled forestry, whilst preparation of land for turbine emplacement may alter the exported DOC to be more resistant. Although the sub-catchment hosting the wind farm (D-WF) is subject to disturbance there is not the residue of younger C in the catchment and so the DOC released here is 'older' and when from deeper peat has a composition of material that has already undergone decomposition. Due to their low nutrient content and sensitivity to water table changing (Nieminen, 2004) organic-rich soils such as peat can be more sensitive to forest activities (e.g. clearfelling) than mineral soils. Further, for many years after the roots can still act as a source of DOC (Hansson et al., 2010).

Whilst concentration varied seasonally, this was not synchronous with pronounced seasonal changes in DOC composition across the Whitelee catchments or within the Drumtee Catchment (WL13). However it was observed that hydrological conditions influenced DOC composition changes, as clear changes were examined in DOC optical properties during dry and wet periods across the Whitelee and within the Drumtee Catchments. The changes that occurred may reflect the solubility of different DOC pools (HAs and FAs) due to the fluctuating water table and the consequent peat soil acidification would change the exported DOC characteristics. Additionally different carbon sources would be transported when water flows through varied soil layers. In Drumtee opposite responses to discharge were apparent in DOC composition between the two streams in July, the dry month, namely the DOC of stream D-WF became less aromatic and humic (smaller $SUVA_{254}$ and $SUVA_{410}$). Moreover, the larger E_4/E_6 for D-WF provided further insight of relatively more FAs than HAs within humic substances pool.

Land use may bring together different key environmental factors as drivers of DOC composition changes that itself may become an overriding factor. Wind farm development (both turbine emplacement and deforestation) on peatlands may lead to long-term [DOC]

increase locally in the draining water. Specifically, deforestation causes greater increases than turbine emplacement, evidenced from the comparison within Drumtee, a small catchment. In addition, wind farm development may also cause spatial difference in DOC composition in Drumtee, with soil disturbance from turbine emplacement releasing more refractory DOC into the stream. An interesting next step would be to consider if these compositional differences affect the fate of C and macronutrients in the fluvial system. In order to understand these potential interactions between DOC and macronutrients (N and P), and key controls on DOC decomposition in a peat-dominated catchment, a further experiment involving biodegradation incubations was designed and is described in Chapter 6.

5 Dissolved [Fe] in peatland catchments and its effect on the biogeochemical cycles of carbon and nutrients

5.1 Abstract

While increased [DOC] in surface water has become a research focus, the links between carbon and other key elements in biogeochemical cycles is still poorly understood. The importance of iron (Fe) in global carbon cycling was overlooked until recently where it was noticed Fe concentration, [Fe], was increasing as was [DOC] in boreal rivers and streams. Fe may alter the mobility and biodegradability of DOM via several biotic and abiotic interactions. The redox reactions of Fe are also suggested to strongly affect the solubility of some nutrients, e.g. phosphate (P), which can have further impacts on DOM biogeochemical cycles in waters. Thus consideration of Fe is needed to understand the potential role of Fe in C and nutrients dynamic cycling in peatland catchments.

This chapter presents the assessment of the dissolved [Fe] in the large Whitelee catchments and the small Drumtee sub-catchments, and reveals there is a significant correlation between [Fe] and [DOC]. Here, relatively greater Whitelee [Fe] was measured compared to other peaty catchments, and similar seasonal trends were observed across all sites. A positive and significant correlation was obtained between [Fe] and [DOC] in all catchments which may emphasise the importance of peat-rich catchments to Fe export to oceans. However, in the driest months, high Fe and C-DOC molar ratio (Fe/DOC) were observed with the potential of DOC precipitation via Fe. This interaction is significant for 1) the consideration of [DOC] export from Fe-rich catchments, 2) the preservation of terrestrial character of peat-derived carbon in fluvial network and 3) the effects on DOC bioavailability in freshwater ecosystems. DOC optical composition was examined to further understand the Fe-DOC interactions in peaty catchments. In all sites, E_2/E_4 (which is inversely related to DOC humification), was found to be significantly negatively correlated with Fe/DOC, suggesting Fe export per DOC was favoured by larger HS proportion. A significant but weak correlation between Fe/DOC and E_4/E_6 (which represents FA:HA) was reported in the Whitelee long-term catchments, suggesting Fe-DOC complexation may be favoured by greater FAs proportion.

Spatial variation of [Fe] and Fe/DOC were observed in this research, with catchment characters including peat cover, land use difference and water retention time acting as the potential controlling factors. In addition, in the Whitelee catchments, [SRP] and [Fe] showed a significant positive relationship ($p = 0.000$, $R^2 = 32$ %), while [TON] and [Fe]

were negatively and weakly-correlated ($p = 0.000$, $R^2 = 23$ %). Thus more [Fe] in stream may indicate greater P but smaller N exports, which can further influence DOC biodegradation.

5.2 Introduction

Iron is a key element, essential for all living organisms due to its involvement in a wide variety of significant metabolic processes (Cornelis and Andrews, 2010). Fe plays an essential role in photosynthetic and respiratory electron transport, nitrate reduction and chlorophyll synthesis during plant metabolism (Sunda and Huntsman, 1995), thus the Fe biogeochemical cycle interacts with biogeochemical cycles of elements such as N and C. Fe is a micronutrient in the oceans which directly supports life in marine systems and subsequently influences the global carbon cycle (Blazevic et al., 2016). Despite Fe being the most abundant element on Earth's crust, Fe concentration, [Fe], is limited in some certain environments such as the open ocean (Turner and Hunter, 2001).

Fe may exist in natural waters such as soil solutions and surface water either as a free (un-complexed) ion or as complexes with both inorganic and organic ligands (Evans, 1989). The level of Fe concentration in surface freshwaters is relatively low, and the European Commission Drinking Water Directive (CEC, 1980) has set the mandatory maximum admissible [Fe] in drinking water at 200 µg l^{-1} (Abesser et al., 2006). However in the last decade an [Fe] increase in surface waters has commonly being observed in upland regions, making Fe concentration and its biogeochemical cycle become a research interest. For example in UK, research has shown that [Fe] in upland waters which were used for drinking water source can be high as up to 1160 µg l^{-1} (Abesser et al., 2006). Positive trends for increasing [Fe] were observed in 27 of 30 rivers draining into the Swedish coast from 1972 to 2010 (Kritzberg and Ekström, 2012), and in the main streams in mid-Wales upland forested catchments [Fe] almost doubled over 23 years (from 1983 to 2006), from around 70 to 120 µg l^{-1} (Neal et al., 2008).

The presence of Fe can increase UV absorption in lake water directly or by interactions with DOC (Maloney et al., 2005). The prevalence of increasing [Fe] has been pointed out as a potential driver of water colour increase and proposed to contribute to the inland waters brownification over large parts of the Northern hemisphere (Kritzberg and Ekström, 2012; Weyhenmeyer et al., 2014), which was previously often considered related to increasing terrestrially derived DOM concentration (Kritzberg and Ekström, 2012). While the DOC biogeochemical cycle has been a research interest over the last decades, its link with other key elements, e.g. Fe, remains poorly understood (Xiao et al., 2016).

Fe is predominantly in solution or in mineral forms in states of Fe^{2+} and Fe^{3+}. It can shift between the two states in fluctuating redox environments, affecting DOM and carbon cycling via biotic/abiotic process. DOM decomposition can be facilitated via microbe-mediated Fe reductive dissolution: 1) labile DOM acts as a source of energy (electrons) for Fe- and HS-reducing bacteria; 2) Fe reduction and DOM are linked through the electron-shuttling ability of HS (Mladenov et al., 2010). To decompose labile DOM, bacteria can transfer electrons from DOM to Fe^{3+}, which works as an electron accepter (Islam et al., 2004). This process allows organic matter oxidation under anaerobic conditions (Li et al., 2012), and the presence of labile DOM can in turn fuel Fe-reduction and subsequent dissolution (McArthur et al., 2004; Mladenov et al., 2010). In addition, DOM decomposition can also be stimulated by abiotic oxidation-reduction reactions, with Fe binding with quinone moieties of DOM. The oxidation of quinone can produce reactive oxygen species and cause breakage of DOM aromatic rings, together leading to facilitate DOM decomposition and produce substrates for microorganisms (Comba et al., 2015; Xiao et al., 2016; Yuan et al., 2016).

Despite the positive effects of Fe on DOM decomposition, Fe may also reduce DOM availability and biodegradability. While Fe and DOM can form dissolved complexes, the reported maximum potential binding capacity for Fe is around 2 umol Fe mg^{-1} C^{-1} (Neubauer et al., 2013). The excess of Fe in solutions can form DOM-Fe precipitates and thus reduce DOM mobility in soil and waters (Nierop et al., 2002). Particularly, the high molar mass organic C coagulates actively with Fe-based coagulants, which are often used in the drinking water treatment research and process (Matilainen et al., 2005; Ritson et al., 2014). Organic C precipitates increased in DOC samples extracted from soils in the Netherlands when the molar ratio between Fe^{3+} and C-DOC (Fe/DOC) was between 0.014 and 0.08 (Nierop et al., 2002). Further, excessive [Fe] can cause decreased bacterial growth and consumption of DOM by formation of insoluble Fe precipitates on bacterial surfaces (Xiao et al., 2016).

In addition to the influence of Fe presence on DOM, DOM can also increase Fe^{3+} solubility by acting as the organic ligand and form stable soluble complexation with Fe (Mladenov et al. 2010; Weyhenmeyer et al. 2014). Water solubility of Fe^{3+} is several orders of magnitude lower than Fe^{2+}, but in surface water the functional groups of DOC e.g. aliphatic and aromatic carboxyl and hydroxyl can readily bind Fe^{3+}, maintaining Fe^{3+} in dissolved state (Maranger et al. 2006; Weyhenmeyer et al. 2014). It has been widely-observed that Fe-DOM complexes are the dominate dissolved Fe speciation exported in fluvial networks (Krachler et al., 2010; Nagai et al., 2007; Neubauer et al., 2013).

HS play a significant role in the transport of Fe in freshwaters with HAs and FAs being strong chelate ligands, and peatland-draining rivers are suggested to be important sources of dissolved Fe to the ocean margins (Krachler et al., 2010), where [Fe] is limited. For example, [Fe] in surface water from Finnish peatland-dominated catchments was 1.92 ± 0.52 mg l^{-1}, but only 0.29 ± 0.12 mg l^{-1} in mineral soil-dominated catchments (Sarkkola et al., 2013). Peat-draining rivers in north Scotland were estimated to contribute about 0.185 mg l^{-1} dissolved iron to the oceans, considerably higher than the dissolved iron concentration of the 'average world' river contribution of only 0.002 mg l^{-1} (Krachler et al., 2010).

Fe is also considered associated with nutrient biogeochemical cycles in soil and aquatic systems, including phosphorus (P) and nitrate (N) (Baken et al., 2015). In Fe-rich systems, reduction and oxidation of Fe determine the mobility and fate of P at the sediment-water interface (Baken et al., 2015). In oxic environments, hydrous Fe oxides readily precipitate and prevent mass mobilization of P into pore water and/or aquatic systems by providing sorption surfaces, yielding a highly efficient P sink (Baken et al., 2013, 2015; Emsens et al., 2016). However high concentrations of organic matter and stable Fe-OM complexes can prevent Fe interactions with P and influent aqueous P concentrations (Sundman et al., 2016). Additionally, P(V)-Fe(III)-OM complexes have been suggested to be an important P pool in soils and are more bioavailable than P in precipitates or bound to inorganic surfaces (Gerke, 2010; Sundman et al., 2016). Associated P can also be released upon reductive dissolution of Fe oxides at the interface between anoxic and oxic environment (Liu, 2010). All these tight associations between Fe, P and organic matter can alter the availability of P to microbial communities and thus potentially influent DOM biogeochemical cycles (Xiao et al., 2016).

The Fe^{2+}-Fe^{3+} reduction/oxidation occurs with/is important to N cycling (Li et al., 2012), and wetland sediments contain microbial communities which are capable of oxidation of Fe^{2+} with reduction of NO_3^- to NH_4^+ (Weber et al., 2006). Nitrate was suggested to interact with Fe biogeochemistry in the subsoil of wetlands, and furthermore immobilizes Fe in the subsoil by oxidizing Fe^{2+} (Li et al., 2012; Smolders et al., 2010).

High [Fe] has been reported commonly in draining systems which are rich in fluvial DOC, e.g. peatland catchments. Given the capacity for Fe to influence and be influenced by other biogeochemical cycles, it is valuable to explore the range of Fe concentrations and flux in the Whitelee peatland catchment, which is rich in strong chelate ligands DOC but limited in nutrients. The main aims of this chapter are to:

1. Assess the temporal and spatial variation in total dissolved Fe concentration ([Fe]) in the small Drumtee sub-catchments and the adjacent larger Whitelee catchments (WL13, 14, 15, 16 and 1). [Fe] time series and estimated specific Fe exports were constructed to quantify Fe transport in small and large carbon-rich peatland catchments.

2. Explore the interaction between Fe and DOC in peatland catchments, by analysing the [Fe]-[DOC] relationship, and the seasonal and spatial variations of Fe-DOC binding ability. DOC composition impacts on these interactions were examined. The obtained information can improve the understanding of Fe and DOC mobilization and biogeochemical cycles. Proper management to improve water quality can also be suggested accordingly in the future.

3. Investigate the association between [Fe] and [SRP], and [TON], to analyse if and how Fe is spatially and temporally related to nutrient biogeochemical cycles in peat-rich catchments. This is important in order to assess the exports and bioavailability of these elements.

5.3 Methods and materials

5.3.1 Measurement of dissolved Fe concentration

The dissolved Fe concentration [Fe], comprising Fe^{2+}, Fe^{3+} and organic-bound Fe, was measured in the water samples collected monthly from April 2014 to February 2016 from five long-term monitoring sites (WL13, 14, 15, 16 and 1) draining the peaty soils of Whitelee wind farm. In sub-catchment Drumtee, monthly [Fe] was also measured in sampling sites along the two tributaries (D-WF and D-FF) during February 2014 to February 2015. Sampling sites were the same as described in Chapter 4, Section 4.3.1).

Samples were filtered according to the same protocol as for [DOC] measurement (Chapter 2, Section 2.3.1.1) and kept in the cold room until measurement. The dissolved [Fe] was measured for filtered samples using the Perkin-Elmer AAnalyst 100 Atomic Absorption Spectrophotometer (AAS). The procedure details are described in the method chapter (Chapter 2, Section 2.3.3).

5.3.2 Estimating monthly Fe flux

Specific monthly Fe fluxes (g m^{-2} $month^{-1}$) for the Drumtee sub-catchments (Feb. 2014 – Feb. 2015) and the Whitelee catchments (Apr. 2014 – Apr. 2016) were estimated by multiplying the instantaneous concentration on sampling days by specific monthly discharge. Discharge in WL13 confluence site was scaled up for the other sites. See Chapter 2 Section 2.4.2 for the details of calculation method.

5.3.3 Data analysis

For Drumtee samples, in order to analyse the spatial variation of [Fe] between the two sub-catchments, sites along each tributary were pooled together in the same protocol as [DOC] analysis (see Chapter 4, Section 4.4.3) to produce a monthly value. This also allows the examination and comparison of any relation between [Fe] and DOC-related parameters. Significant difference between the Whitelee long-term sites, or between Drumtee sub-catchments were examined by the Tukey Test in ANOVA ($p < 0.05$ was considered as significantly different).

Stream [DOC], [SRP], [TON] and UV-visible optical characteristics of DOC, discussed previously in Chapter 4, were investigated for their relationships with [Fe]. The molar ratios between dissolved Fe and C-DOC, and SRP (hereafter referred as Fe/DOC and Fe/P respectively) were calculated. Fe/DOC was used as a predictor for the likelihood of DOC complexation of Fe, and DOC precipitates would be expected when Fe/DOC was greater than 0.014 (Nierop et al., 2002). Simple linear regressions were examined between [Fe] and [DOC], [SRP] and [TON] (noting nutrients were only measured at long-term sites). UV-visible optical characteristics were analysed for correlation with Fe/DOC molar ratio. However only E_2/E_4 and E_4/E_6 were considered because SUVA data would be potentially correlated with Fe/DOC, as it is indicating absorbance per unit of DOC. Relationships were considered significant when $p < 0.05$.

5.4 Results

In the following sections (5.4.1 and 5.4.2), Drumtee sub-catchments are considered first and then the Whitelee catchments.

5.4.1 [Fe] and its correlation with [DOC]

5.4.1.1 Drumtee sub-catchments

Drumtee [Fe] showed little variation within tributary sites on each sampling date (intra-site SD ranged from 0.06 to 0.58 in D-WF, 0.08 to 1.27 in D-FF) (Fig. 5-1), indicating the approach to pooling data was appropriate. However, differences in [Fe] were observed between the two tributaries. [Fe] in D-WF over the year ranged from 0.19 ± 0.07 – 2.98 ± 0.58 mg l^{-1}, and in D-FF from 0.35 ± 0.08 – 3.99 ± 1.27 mg l^{-1} (Fig. 5-1); the yearly average concentration in D-FF (2.10 ± 1.13 mg l^{-1}) was almost twice high as that in D-WF (1.22 ± 0.78 mg l^{-1}). ANOVA analysis indicated the difference in [Fe] between D-WF and D-FF was significant ($P < 0.05$). Strong and similar seasonal trends were observed in both streams, with higher concentrations in the summer period, and a maximum in September, before decreasing in late autumn and winter, with the smallest concentration both in January.

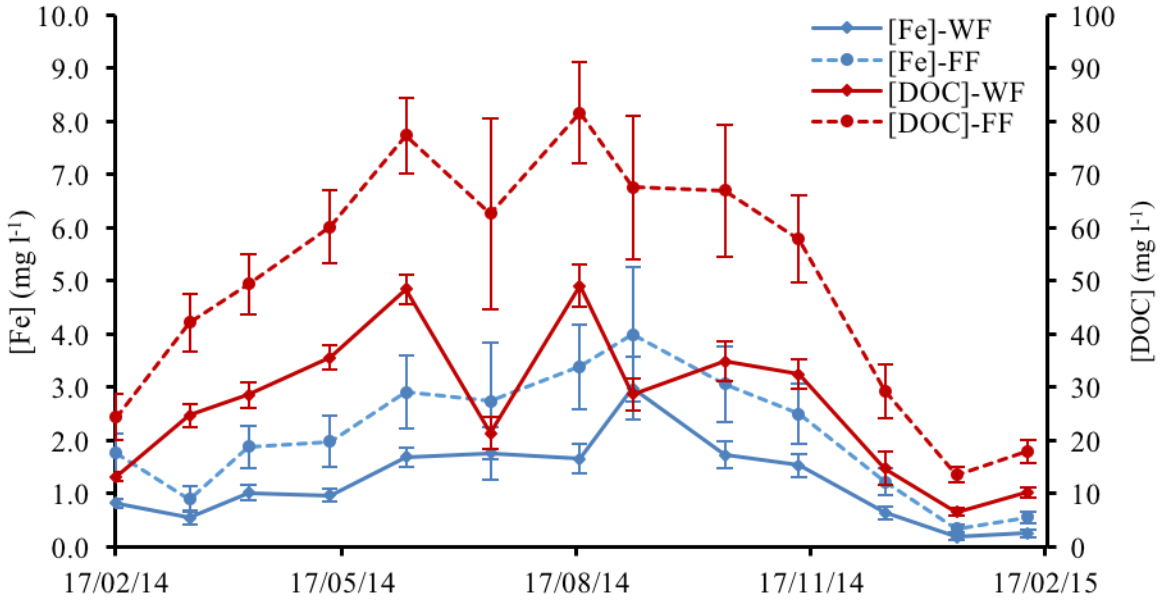


Fig. 5-1. The dissolved [Fe] and [DOC] in Drumtee streams from February 2014 to February 2015. Mean values of sampling sites along each stream are presented with the standard deviation showing the intra-stream variation. Concentrations in D-WF are indicated in solid lines and D-FF in dash lines.

[DOC] and [Fe] co-varied in most months (Fig. 5-1), and shared a similar seasonal pattern, exhibiting higher concentrations in the summer period whilst the smallest in winter time. However, it is noticeable that in July and September 2014, the drier months, there were decreases in [DOC] in both streams while [Fe] remained stable in July and increased in September.

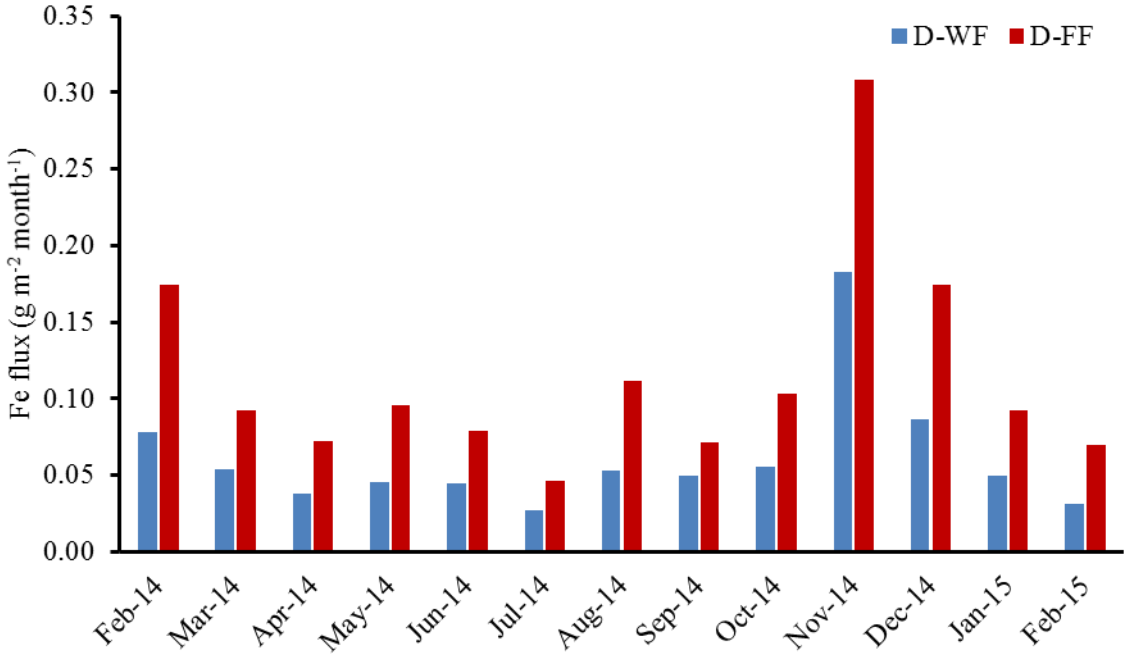


Fig. 5-2. Specific monthly Fe fluxes in Drumtee streams from February 2014 to February 2015. Both showed a similar autumn flush. Fe and DOC fluxes in D-WF are indicated in solid lines and those in D-FF are in dashed lines.

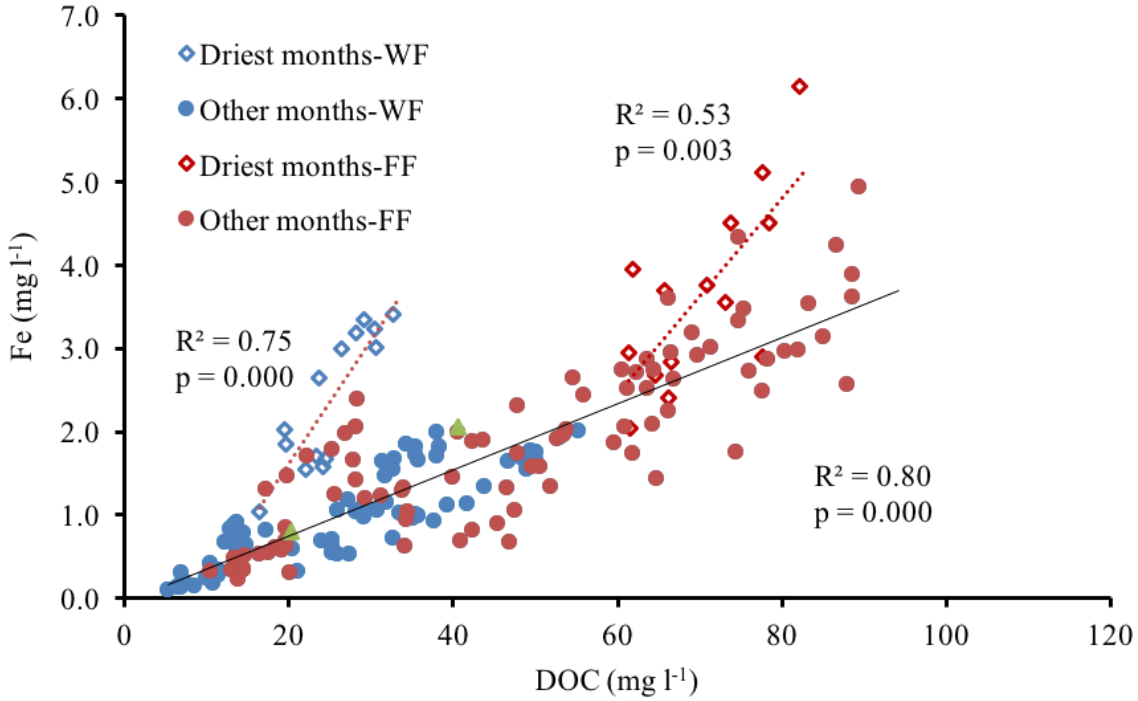


Fig. 5-3. Relationships between [DOC] and [Fe] of samples from all 15 sites in Drumtee from February 2014 to February 2015. The dataset was divided into four groups according to the sampling period (the driest months or the other months) and streams (D-WF and D-FF). The black solid line is the trend line for both D-WF and D-FF excluding samples from the driest periods (July and September 2014). Samples in the driest months are represented by empty symbols, while the rest by full symbols. The two green triangle symbols represent the outliers from the dry-FF group, and are not considered for any relationship.

Each month more Fe was exported from D-FF (0.11 ± 0.07 g m^{-2} $month^{-1}$) than from D-WF (0.06 ± 0.04 g m^{-2} $month^{-1}$) ($p < 0.05$) (Fig. 5-2). The fluxes in both streams shared the same seasonal pattern, showing a decrease from spring to summer (March to July),

regardless of the generally increasing trend of [Fe] during the same period. Fe fluxes of both streams showed a large autumn flush in November resulting in a maximum flux in both D-WF and D-FF (D-WF: increased to 0.18 g m^{-2} $month^{-1}$; D-FF: to 0.31 g m^{-2} $month^{-1}$) and a gradual decrease into winter.

Correlations between [DOC] and [Fe] were examined for all Drumtee samples. In general, [Fe] was positively-correlated with the whole sample [DOC] population (Fig. 5-3). However, samples collected during the driest months of the monitoring (July and September 2014) were separated from the samples in the other months (see Chapter 4 Fig. 4-6 for hydrograph). Positive trends were seen in the driest months, but with higher [Fe]. When all samples in the driest periods were excluded, the rest of samples from both streams showed a significant and stronger relationship between [Fe] and [DOC]:

$$[Fe] = 0.040 * [DOC] - 0.036 \ (R^2 = 0.80, p = 0.000)$$

Significant correlations were also observed for the driest months' samples: in D-WF p = 0.000, R^2 = 0.75; in D-FF p = 0.003, R^2 = 0.53.

5.4.1.2 Whitelee catchments

[Fe] was also measured for a longer period, in the five long-term sites of the Whitelee catchments, from 10/04/2014 to 19/09/2016. A similar seasonal trend to that in Drumtee was observed in all five sites: the smallest [Fe] occurred in winter and the greatest in late summer or early autumn (Fig. 5-4 upper). [Fe] ranged from 0.15 – 3.57 mg l^{-1}, with the smallest concentration in WL1 in January 2015 and the greatest in WL15 in September 2014. Spatially the average [Fe] among the sites followed this order: WL15 (1.70 ± 0.86 mg l^{-1}) > WL16 (1.49 ± 0.79 mg l^{-1}) > WL13 (1.33 ± 0.70 mg l^{-1}) > WL1 (1.17 ± 0.59 mg l^{-1}) > WL14 (0.88 ± 0.38 mg l^{-1}). Inter-site variation was greatest in summer and early autumn and least in winter. In the summer of 2014, the changes in [Fe] lost synchronicity: in July [Fe] in both WL15 and 16 increased, whilst the other sites all showed a decrease, and it was the other way round in August.

With the assumption that specific water discharge was similar among different Whitelee catchments, specific monthly Fe flux in each long-term site was estimated according to discharge monitored at WL13 (Fig. 5-4 lower). Mean values of specific monthly Fe exports ranged from 0.07 ± 0.05 g m^{-2} $month^{-1}$ in WL14 to 0.13 ± 0.07 g m^{-2} $month^{-1}$ in WL15. The exports were similar among most sites, but significantly different between WL14 and WL15 ($p < 0.05$). Similar changing patterns were seen among all sites, while variations were observed among years (Fig. 5-4 lower). In 2014 the specific fluxes were relatively

constant and small in spring and summer periods, before a large increase in late autumn (November) which gradually decreased into the following spring. The similar large increases in Fe exports in the late autumn also occurred in 2015, while fluxes also showed peaks during the summer periods.

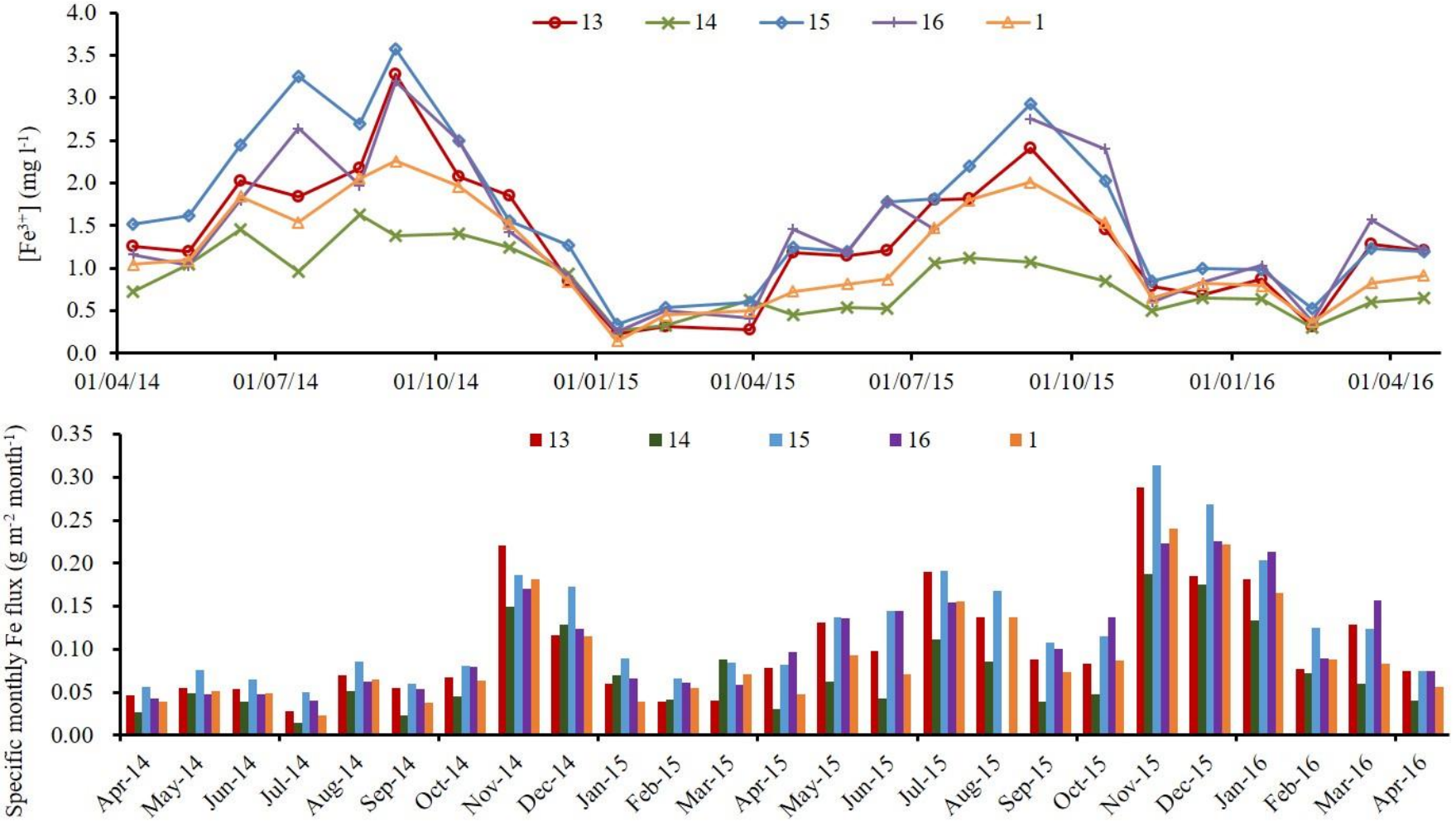


Fig. 5-4. The dissolved [Fe] (upper) and specific monthly Fe fluxes (lower) of long-term sites in the Whitelee catchments from April 2014 to April 2016. WL13 is the confluence site located in Drumtee. In Aug. 2015, there was no data for WL16 as the sampling site was inaccessible due to road construction. It was assumed when calculating export that the concentration of the last sample carried for longer.

The significant correlations between [Fe] and [DOC], observed in Drumtee tributaries, also existed in the large catchments (p = 0.000), with the driest months (July, Sept. 2014 and Sept. 2015, see Chapter 4 Fig. 4-6 for hydrograph) having a different, but still significant relationship (p = 0.009) (Fig. 5-5). However it was also shown that driest-period samples plot off the bulk sample trend line, along with a few other samples from the rest periods, for example WL16 in October 2014 and March 2015.

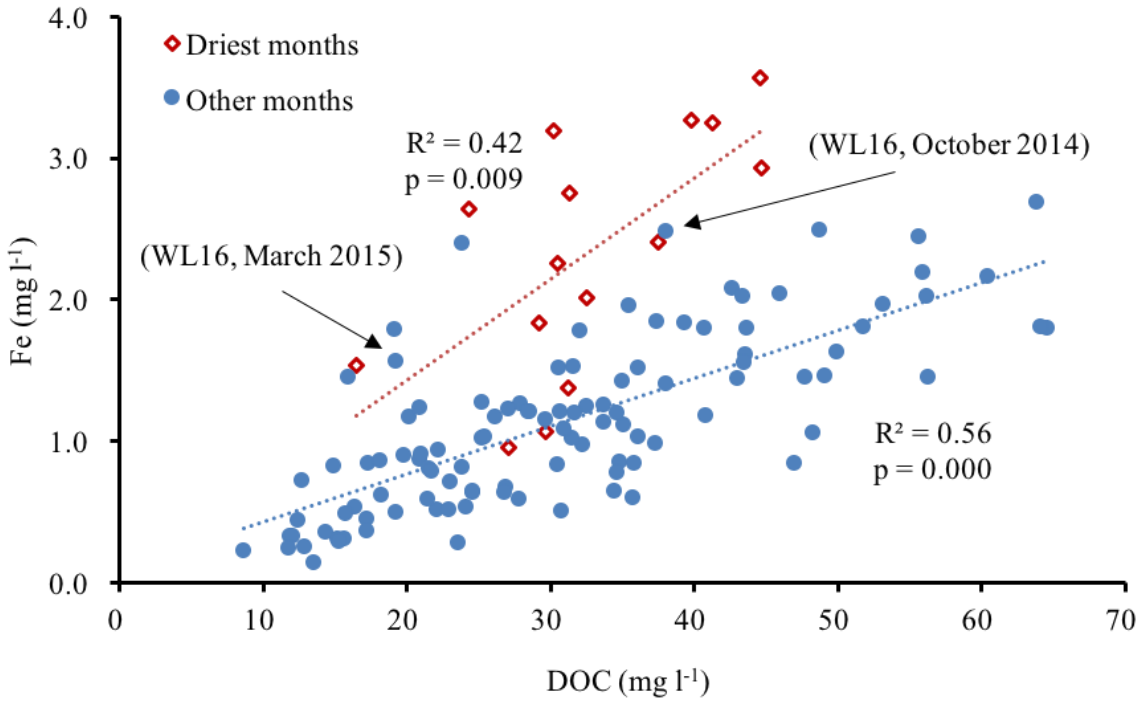


Fig. 5-5. Relationships between [DOC] and [Fe] of samples from all Whitelee long term sites (WL13, 14, 15, 16, 1) from April 2014 to April 2016. The driest months are July, September 2014 and September 2015.

Table 5-1 Relationships between [DOC] and [Fe] in sub-catchments D-WF, D-FF in Drumtee, and in the Whitelee catchments (WL).

Hydrological condition	Sites	Relationships
Other months	D-WF	$y = 0.035x + 0.046$ ($R^2 = 0.77$, $p = 0.000$)
	D-FF	$y = 0.040x - 0.041$ ($R^2 = 0.74$, $p = 0.000$)
	WL	$y = 0.034x + 0.096$ ($R^2 = 0.56$, $p = 0.000$)
Driest months	D-WF	$y = 0.148x - 1.331$ ($R^2 = 0.75$, $p = 0.000$)
	D-FF	$y = 0.117x - 4.554$ ($R^2 = 0.53$, $p = 0.003$)
	WL	$y = 0.071x + 0.018$ ($R^2 = 0.42$, $p = 0.009$)

The [Fe]-[DOC] relationships for sub-catchments (D-WF and D-FF) and large Whitelee catchments are more similar in the non-driest periods ('Other months' is Table 5-1). Fe and DOC were more strongly correlated in the tributaries during the driest months, but

not in the large catchments. For the consideration of Whitelee catchments, the relationship was weaker in the driest months (R^2 < 0.50) than the rest of the time (R^2 > 0.50).

5.4.2 DOC composition impacts on Fe-DOC binding

5.4.2.1 Drumtee sub-catchments

For most months, Fe/DOC in both streams were similar, and smaller than 0.014. Generally there were larger Fe/DOC values in summer/autumn than spring/winter for two Drumtee streams during 2014 – 2015, apart from a large Fe/DOC in February 2014 (a wet month: D-WF: 0.013 ± 0.001; D-FF: 0.016 ± 0.002) (Fig. 5-6). In July and September 2014 (the dry months), Fe/DOC in D-WF increased to be much larger than 0.014 (July: 0.018 ± 0.004; September: 0.022 ± 0.003). This was not observed with D-FF Fe/DOC bar a small increase in September, and this ratio remained smaller than 0.014.

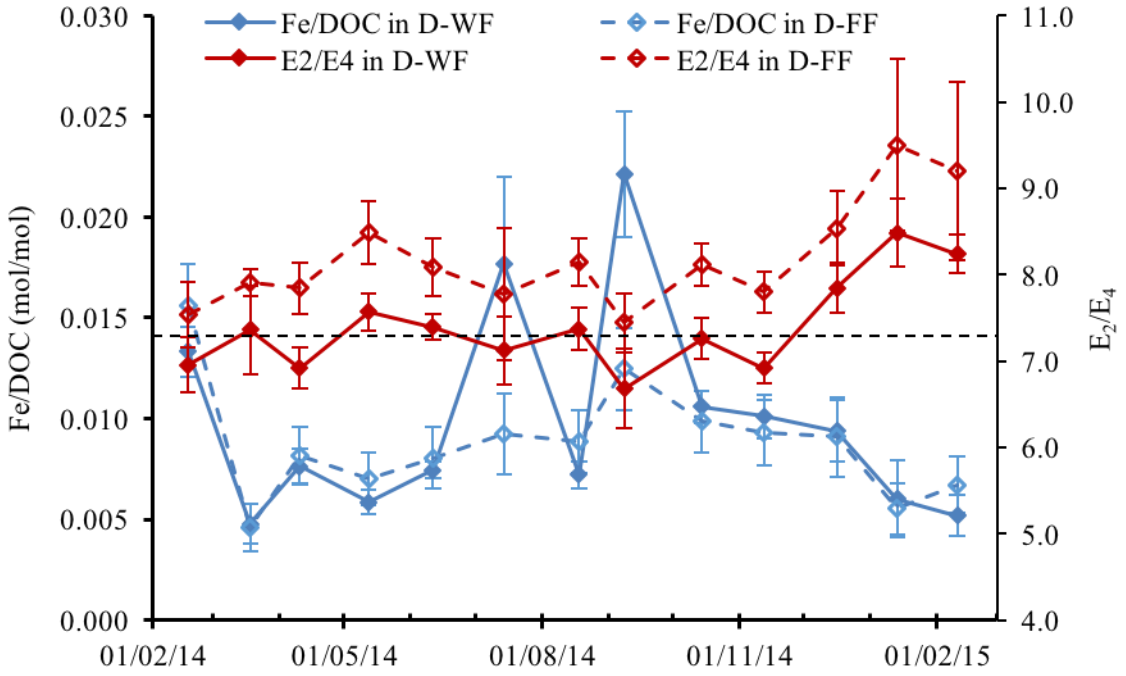


Fig. 5-6. Molar ratio Fe/DOC and E_2/E_4 in Drumtee streams from February 2014 to February 2015. Ratios in D-WF are indicated in solid lines and those in D-FF are in dashed lines. The black dashed line represents Fe/DOC of 0.014 as a point when DOC precipitates may be observed (Nierop et al., 2002).

During this time period, E_2/E_4 showed temporal changes broadly opposite to Fe/DOC (Fig. 5-6). This interaction was less clear with E_4/E_6 (so the plot of contemporaneous temporal changes is not given but the interaction plot is Fig. 5-7 lower). Correlation results for all Drumtee sites revealed E_2/E_4 was significantly correlated with Fe/DOC, showing a negative relationship in both sub-catchments regardless the climatic differentiation (Fig.

5-7 upper). The relationship between Fe/DOC and E_2/E_4 was for 'Other months-WF', 'Other months-FF' and 'Driest months-FF', while 'Driest months-WF' samples fell off the main trend having a higher Fe/DOC ratio. The relationship between Fe/DOC and E_2/E_4 overlapping samples (all groups except 'Driest months-WF') was generally weak, explaining only 13 % of variations in Fe/DOC changes, but for D-WF during the driest periods the relationship was stronger with $R^2 = 0.85$.

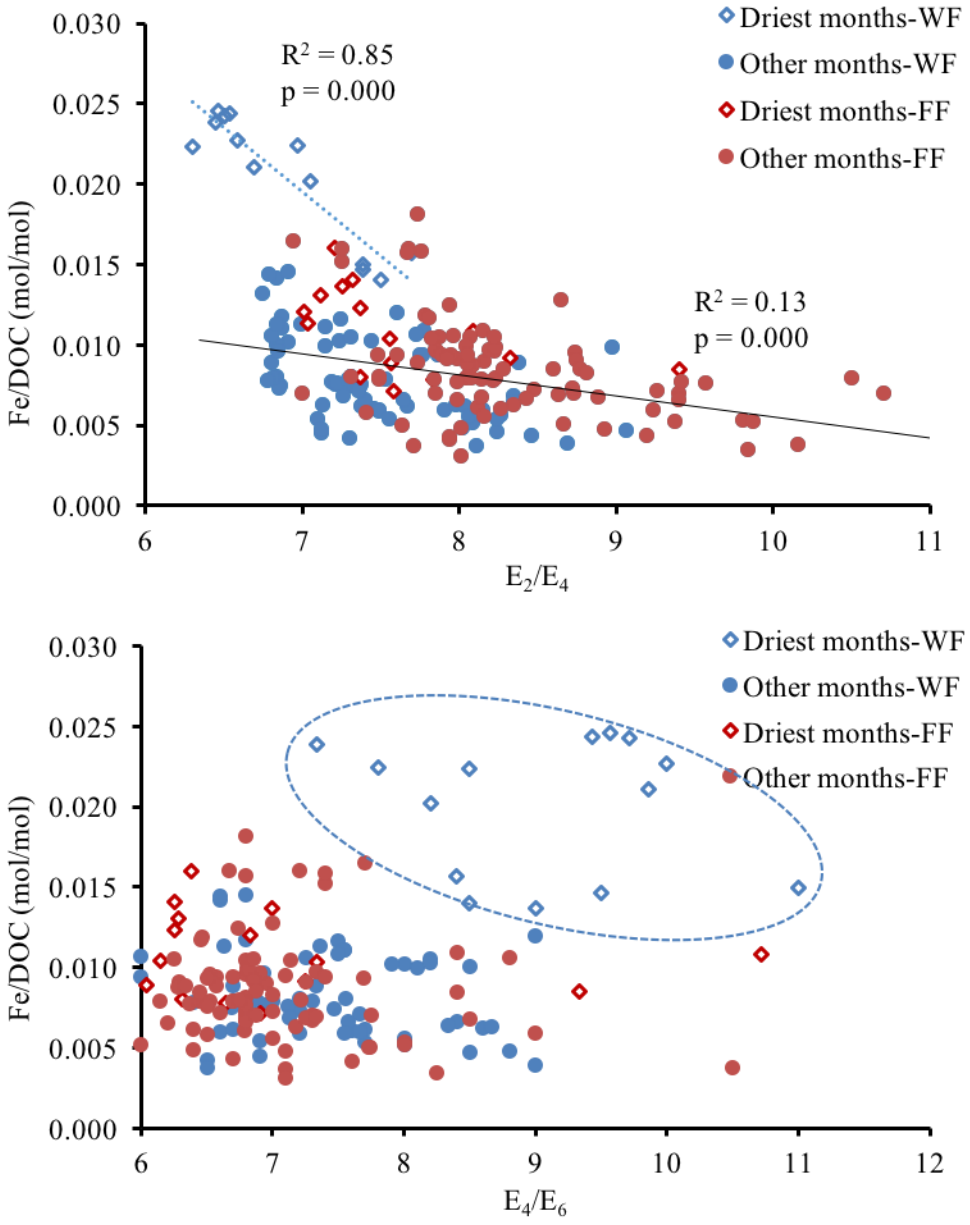


Fig. 5-7. Relationships between E_2/E_4 and Fe/DOC (upper), E_4/E_6 and Fe/DOC (lower) of samples from February 2014 to February 2015 for all 15 sites in Drumtee. The dataset was divided into four groups according to the sampling period (the driest months or other months) and streams (D-WF and D-FF). The black solid line in upper graph represents the linear trend line of Other months-WF, Other months-FF and Driest months-FF. No significant correlation was generated for the relationships between E_4/E_6 and Fe/DOC.

Correlations between E_4/E_6 and Fe/DOC were not statistically significant in all cases ($p > 0.05$), therefore these interactions are not shown. However once again it was observed

that D-WF samples from the driest months were generally separated from the rest overlapping samples (Fig. 5-7 lower).

5.4.2.2 Whitelee catchments

Fe/DOC molar ratio in the Whitelee catchments ranged from 0.002 to 0.023 during 2014 – 2016, and for most of the time was smaller than 0.014 (Fig. 5-8). A more constant and smaller Fe/DOC was observed in WL14 than the other sites which were variable and larger. The averaged Fe/DOC among sites followed this order: WL16 (0.011 ± 0.006) > WL1 (0.009 ± 0.004) > WL15 (0.009 ± 0.003) > WL13 (0.008 ± 0.003) > WL14 (0.006 ± 0.002). Fe/DOC in WL14 was significantly smaller than that in WL16 and WL1 ($p < 0.05$), while the difference between WL13 and WL16 was also significant ($p < 0.05$).

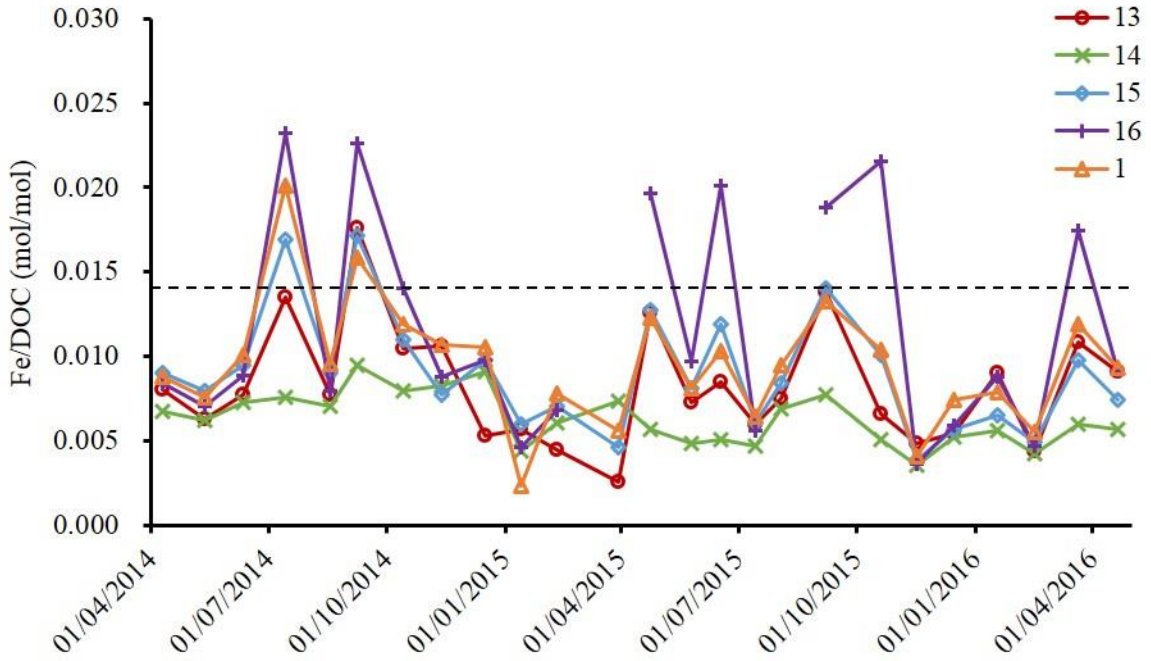


Fig. 5-8. Molar ratio Fe/DOC in Whitelee long-term sites from April 2014 to April 2016. The black dashed line represents Fe/DOC of 0.014 as a point when DOC precipitates may be observed (Nierop et al., 2002). In Aug. 2015, there was no data for WL16 as the sampling site was inaccessible due to road construction.

Generally Fe/DOC values across Whitelee were smaller in early spring and higher in summer/autumn (Fig. 5-8), consistent with the observation in Drumtee sub-catchments (Fig. 5-6). The ratios in the driest months being greater than or larger to 0.014, which occurred in Drumtee D-WF, also existed in Whitelee (July and September 2014 and September 2015) for all except WL14 (July 2014: 0.008; September 2014: 0.009; September 2015: 0.008). Fe/DOC also increased in April and June 2015, and March 2016, which were wet months, but only WL16 had a ratio greater than 0.014.

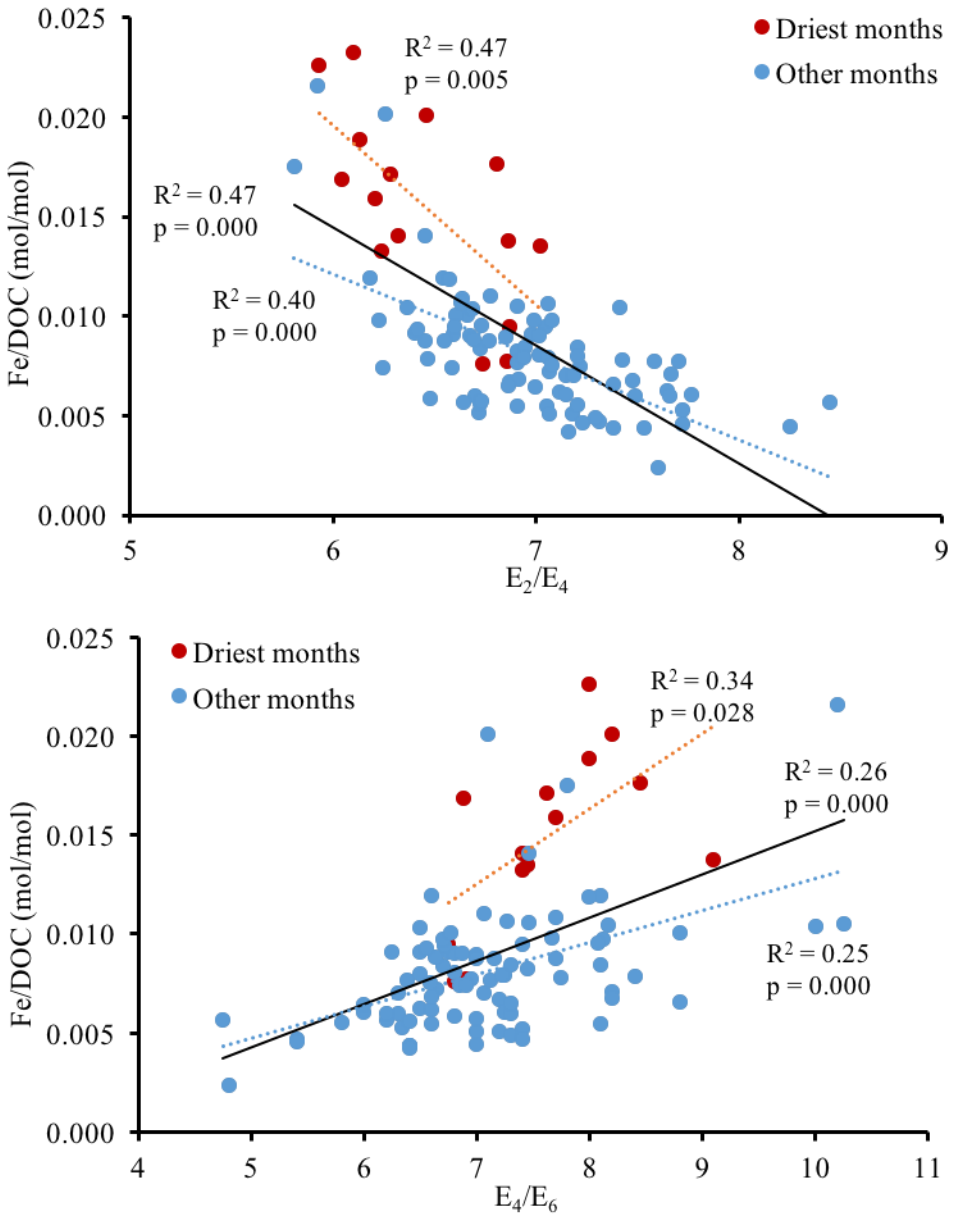


Fig. 5-9. Relationships between E_2/E_4 (upper), E_4/E_6 (lower) and Fe/DOC of samples from all Whitelee long term sites from April 2014 to April 2016. The dataset was divided into two groups according to the climatic conditions (the driest months or other months). The driest periods are July, September 2014 and September 2015. The red dashed line is the trend line for 'Driest months' samples, blue for 'Other months' ones and black for all samples regardless of the climatic differentiation.

Relationships between E_2/E_4, E_4/E_6 and Fe/DOC were also considered for all Whitelee samples. E_2/E_4 and Fe/DOC were significantly and negatively correlated for samples from both the driest ($p = 0.005$) and the other months ($p = 0.000$), and such so for all samples regardless of climatic differentiation ($p = 0.000$, Fig. 5-9 upper). Stronger relationships were shown in large Whitelee catchments than that in small sub-catchments in Drumtee (Fig. 5-7 upper, excluding Driest months-WF), with E_2/E_4 explaining similar and larger variance in Fe per unit DOC (R^2 ranged from 0.40 to 0.47). The slope of trend line in the driest period was steeper than the remainder of the time.

Significant and positive relationships were observed in E_4/E_6 and Fe/DOC for all samples in Whitelee, and those in the driest and the other periods (Fig. 5-9 lower). However these relationships were weak, explaining around 25 – 34 % of variation of Fe/DOC changes in different groups. Once again, the driest-period samples were outside the main group with a few samples from the other period, similar to the observations in E_2/E_4 and Fe/DOC relationship.

5.4.3 Interaction between Fe and nutrients: Whitelee catchments

Similar to the [DOC]-[Fe] relationship, [SRP] and [Fe] were also positively-correlated across the five Whitelee sites and different climatic conditions (the driest and the other months) (Fig. 5-10). The relationship for all sites was significant (p = 0.000) although [Fe] alone only explained 32 % of the variations in [SRP] changes. A negative and curvilinear relationship with [Fe] was observed for [TON] (Fig. 5-11). As with [SRP], no obvious difference seemed to exist for different climatic conditions, and the correlation between [Fe] and [TON] was weaker (R^2 = 24 %) than that in the [Fe]-[SRP] relationship.

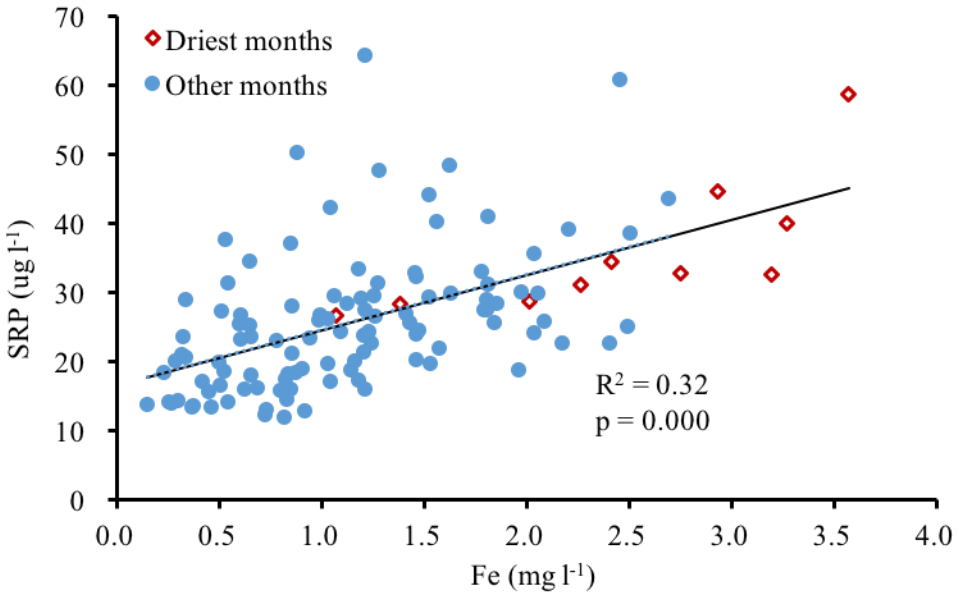


Fig. 5-10. The relationship between [Fe] and [SRP] of samples from the Whitelee long-term sites (WL13, 14, 15, 16, 1) from April 2014 to April 2016. The driest months are July, September 2014 and September 2015. The solid black line is the relationship for all samples.

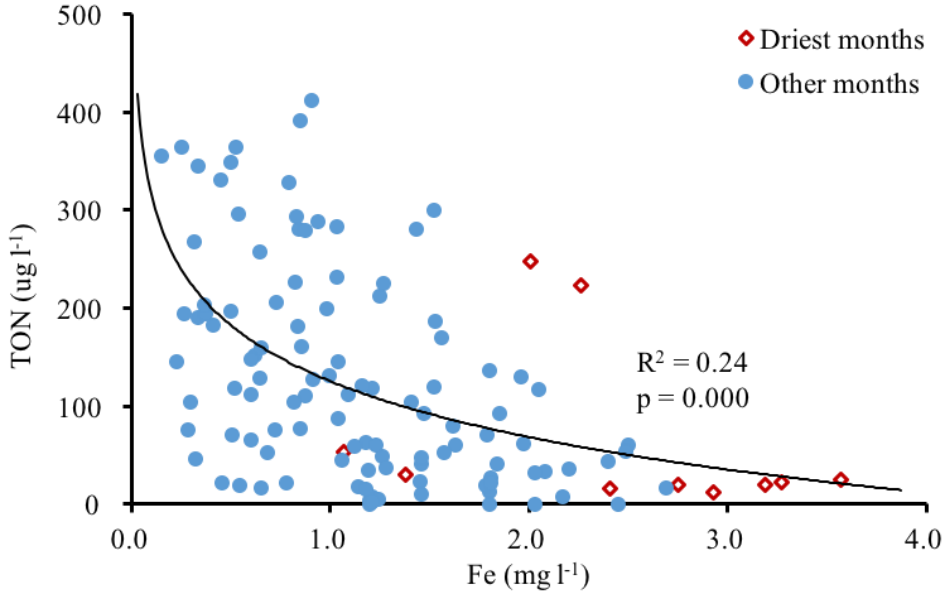


Fig. 5-11. The relationship between [Fe] and [TON] of samples from all Whitelee long term sites (WL13, 14, 15, 16, 1) from April 2014 to April 2016. The driest months are July, September 2014 and September 2015, but no [TON] measured for July 2014 as a practical mistake. The solid black line is the relationship for all samples.

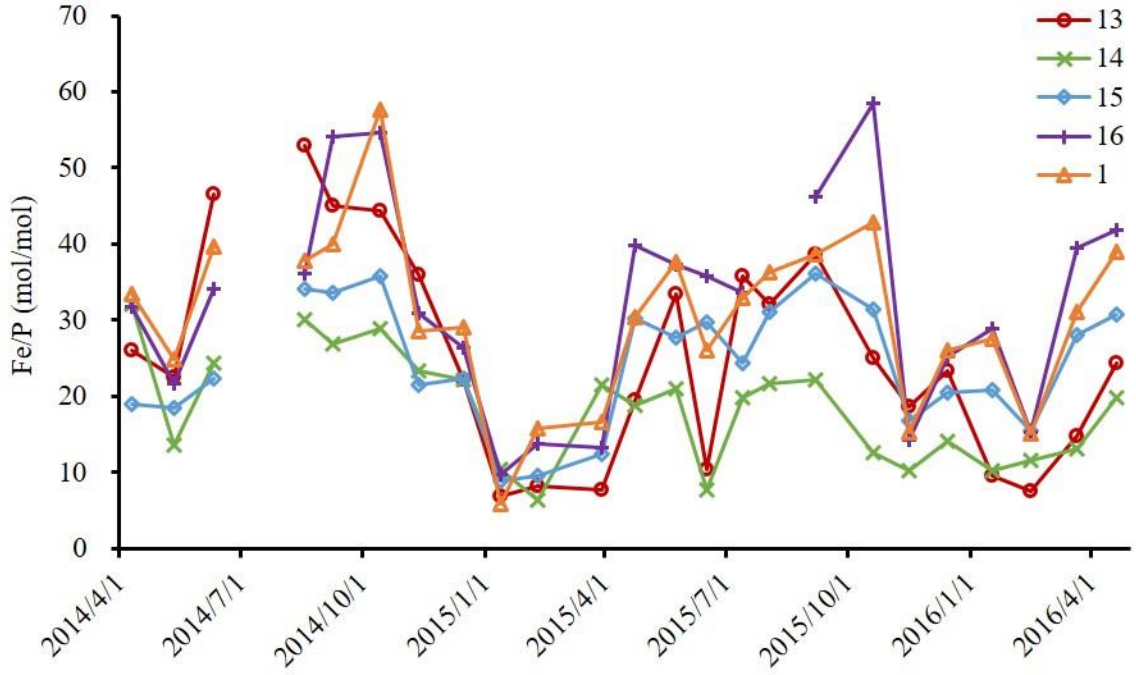


Fig. 5-12. Molar ratio Fe/P in Whitelee long-term sites from April 2014 to April 2016. No Fe/P data for July 2014 as nutrient was not measured on that sampling date.

The molar ratio Fe/P across the Whitelee catchments ranged from 5.9 (WL1 in January 2015) to 58.4 (WL16 in October 2015) (Fig. 5-12) during April 2014 – April 2016. A general seasonal pattern in Fe/P was exhibited, with the greater ratios in the summer and decreasing through autumn to winter. The averaged Fe/P was largest in WL16 (32.3 ± 13.8), followed by WL1 (30.3 ± 11.3), WL13 (25.5 ± 13.8) and WL15 (24.2 ± 8.14). Fe/P in WL14 was the smallest (18.4 ± 7.33) and significantly smaller than WL16 and WL1 ($p < 0.05$).

5.5 Discussion

5.5.1 High [Fe] in peatland catchment and interaction with DOC

Soils and waters in peat-dominated catchments tend to deliver higher [Fe] than catchments with mineral and gley soils (Table 5-2). The [Fe] in streams in this study was all in excess of 200 ug l^{-1} across the years (only apart from January 2015), which is the maximum admissible [Fe] in drinking water (Abesser et al., 2006). Whitelee [Fe] was similar to peatland-dominated catchments in Finland and relatively higher than those from other peaty catchments (including some in the North Scotland), but not as high as some soil water and groundwater sites that seem to have the highest [Fe] (Table 5-2). The dissolved [Fe] in all Whitelee sites showed seasonal patterns with highest concentrations normally in summer with lower stream flow (Fig. 5-1 and Fig. 5-4). High discharge-induced anaerobic conditions in the catchment was suggested to likely increase Fe transport to fluvial systems (Ekström et al., 2016), which however did not happen here. This may suggest other catchment characters may control [Fe] seasonality in peaty waters.

In addition to the rocky parts of Earth being the initial and main source for Fe in catchments (Rauch and Pacyna, 2009), in streams and rivers, both deep soil water/groundwater and organic soil water are found to be the major source for Fe. The Fe mobilisation from organic-rich soil is generally related to the binding with organic ligand (e.g. DOC) (Mladenov et al. 2010; Weyhenmeyer et al. 2014). The strong and positive correlation between total Fe and DOC concentrations in Drumtee and Whitelee stream water is similar to that observed previously elsewhere, including stream surface water, pore water and sediments (e.g. Knorr, 2013; Oni et al., 2013). Indeed, the high concentration and seasonality of dissolved Fe in the aerobic streams and rivers in this research may be influenced by the high [DOC] exported from peat-dominated soils. The correlation analysis may indicate that between 42 – 80 % of the dissolved Fe across sites was with DOC-chelates, with this range reflecting different hydrological flow pathways that are climatically responsive (Fig. 5-3 and Fig. 5-5). The availability of DOC may be particularly important in supporting Fe^{3+}. Increased DOM-Fe complexation has been reported to be the mechanism behind increasing Fe trends (Sarkkola et al., 2013). As Fe^{3+} is of several orders of magnitude lower water solubility than Fe^{2+}, the binding with DOM and formation of organometallic complexes significantly increase Fe^{3+} stability and dissolved [Fe] in water, and is important for Fe mobilization (Ekström et al., 2016; Krachler et al., 2010).

Table 5-2 Summary of dissolved [Fe] in different environments including river water, stream water, groundwater and soil water. The range of [Fe] in Whitelee Catchment includes concentrations observed in both long-term and short-term sites. The referred examples are listed according to highest [Fe] in each sample (maximum [Fe] was considered).

Samples	[Fe] (mg l^{-1})	Reference
Mainstreams in forested catchments in mid-Wales	0.09 – 0.12	(Neal et al., 2008)
Peat and podzolic soil dominated catchments in mid-Wales	0.01 – 0.14	(Neal et al., 2008)
Deep groundwater from Germany wetland	0.24 (max)	(Knorr, 2013)
Surface water from mineral soil-dominated catchments in Finland	0.29 ± 0.12	(Sarkkola et al., 2013)
Gley soil dominated catchments in mid-Wales	~ 0.90	(Neal et al., 2008)
River water from peat-draining rivers in North Scotland	0.73 – 1.07	(Krachler et al., 2010)
Stream water from a Swedish headwater catchment draining forest and mire	1.24	(Oni et al., 2013)
Surface water from black rivers and peat bogs in Siberia	0.03 – 1.40	(Pokrovsky et al., 2006)
Surface water from Germany wetlands	0.00 – 1.50	(Knorr, 2013)
Surface water from 30 Swedish rivers with different soil types	0.08 – 1.72	(Kritzberg and Ekström, 2012)
Surface water from peatland-dominated catchments in Finland	1.92 ± 0.52	(Sarkkola et al., 2013)
Surface water from forested Swedish catchment with < 10 % peatland	0.21 – 1.98	(Ekström et al., 2016)
Stream water from peatland-dominated Whitelee catchments in Scotland	*0.15 – 4.27*	*This study*
Stream water from peatland-dominated Drumtee sub-catchments in Scotland	*0.19 ± 0.07 – 4.27 ± 1.08*	*This study*
Soil water from post-fell peaty gley in mid-Wales	0.12 – 5.03	(Neal et al., 2008)
Wetland groundwater in German	0.20 – 5.90	(Knorr, 2013)
Groundwater in upland catchments in mid-Wales	0.00 – 7.18	(Neal et al., 2008)
Wetland pore water from a Germany minerotrophic fen site	0.10 – 20.40	(Goldberg et al., 2010)

There are two areas of significance to further consider in relation to the Fe-DOC interaction. The first is that DOC composition may enhance Fe export. The second is that Fe may limit DOC export. Both may occur at the same time and interact and the evidence for this at Whitelee is explored in the discussion that follows.

DOC composition may enhance Fe export: Changes in DOC composition may also influence the association between Fe and DOC. The negative relationship between E_2/E_4 and Fe/DOC molar ratio (Fig. 5-7 and Fig. 5-9) reveals more Fe was mobilized per unit DOC when DOC had larger proportion of HS. This is consistent with the previous suggestion that complexation bindings occurred between Fe and organic ligands particularly with aromatic structure, and adsorptive processes with Fe oxyhydroxides strongly favour the accumulation of the more recalcitrant DOM (Kaiser and Guggenberger, 2000; Kritzberg et al., 2014; Riedel et al., 2013). This may be one potential mechanism contributing to the larger Fe/DOC during low flow phase. While [DOC] decreased in the driest months, [Fe] maintained stable or increased in the streams (Fig. 5-1), which may due to Fe-rich deep soil water/groundwater becoming dominant during low flow periods and supplying more dissolved Fe (Abesser et al., 2006). In addition, the greater DOC binding ability would be expected when DOC with a larger proportion of HS was exported from deeper organic soil (indicated by decreased E_2/E_4, Fig. 5-6), and support the Fe^{3+} in dissolved state, which would otherwise precipitate at the oxic surface.

However, in Whitelee catchment samples, a positive relationship was observed between Fe/DOC and E_4/E_6 (the FA:HA indicator) (Fig. 5-9). The higher the value of E_4/E_6 the greater the proportion of FAs, suggesting that here more Fe was mobilized per unit DOC when it contained less HAs. The results seem to contradict one another. However HAs have larger molecular weight and aromaticity, which could make them more prone to aggregate (Kritzberg et al., 2014) with Fe. Thus in Whitelee FAs might accelerate more close complexation interaction with Fe.

High [Fe] may limit DOC export: It has been suggested that instead of DOC being a regulator of Fe concentration, DOC exports are mediated by co-precipitation with Fe oxides (Knorr, 2013). For example, at the redox interfaces in peatlands, a large portion of DOM (27 %) was removed via coagulation when precipitation of more than 90 % of iron hydroxides happened at the oxic surface (Riedel et al., 2013). It is possible this too occurs in the Whitelee catchments. In some months, particularly in the summer periods, while DOC showed greater binding ability, the Fe/DOC (of filtered water samples) was close to or larger than 0.014 (Fig. 5-1), which may suggest a reduced potential binding sites for Fe^{3+} and thus a greater potential for DOC to precipitate with Fe^{3+} oxides during transport.

Consequently, when humic DOC increased Fe export during low flow phase, greater Fe source from deep peat water/groundwater may cause particle organic carbon to form and DOC to be removed from flow. Thus, the decreased [DOC] in the driest months may be regulated not only by the reduced flush of DOC due to low hydrological flow, but also by the C precipitates with more Fe from deeper peat.

This is significant for three reasons. Firstly, if such precipitation had taken place, then it may provide another mechanism behind organic carbon loss in fluvial networks before reaching oceans (Chapter 1). Secondly, this coagulation of Fe with DOM has been suggested to shelter and preserve organic carbon (Kaiser and Guggenberger, 2000; Lalonde et al., 2012), and so Fe may play an important role in carbon cycles and the export of POM to marine systems that preserves a terrestrial character. Thirdly, this precipitation can reduce DOC availability to in-stream biodegradation.

5.5.2 Potential factors leading to the spatial variation of [Fe]

Hydrological response may influence inter-catchment/sub-catchment homogeneity and Fe export seasonality, however given the catchments are small and close together this is unlikely to differ much across catchments and so cannot explain inter-catchment/tributary differences. Thus other catchment characteristics may be important controls here.

Peat soil cover in the catchment: As peat produces strong ligands (HS) which forms Fe-DOC complexes, the difference of peat coverage (%) in each catchment would be expected to influence the spatial variation of [Fe] across Whitelee. Indeed, the larger averaged [Fe] during 2014 – 2016 was observed in WL15 (1.70 ± 0.86 mg l^{-1}), WL16 (1.49 ± 0.79 mg l^{-1}) and WL13 (1.33 ± 0.70 mg l^{-1}), which had the similar and greater peat cover (90.0 %, 82.0 % and 88.0 % respectively, c). When smaller area of WL14 and WL1 was covered by peat-related soil (78.6 % and 62.0 % respectively), correspondingly there was lower averaged [Fe] in both catchments (WL1: 1.17 ± 0.59 mg l^{-1}; WL14: 0.88 ± 0.38 mg l^{-1}).

Land use difference: However, peat cover cannot fully explain the spatial variation of [Fe], e.g. in Drumtee, there was similar peat cover between the sub-catchments. Land use has been suggested to change the source for DOC and Fe to fluvial systems (e.g. Muller et al., 2015). Turbine installation and the relevant construction were one of the direct land uses in Whitelee. However, little Fe release would be expected from this process into the system, as it would only happen when the turbine foundation was weathered,

and this was not the case in this field. Instead, impacts of wind farm development and the associated felling activities on DOC concentration in the Whitelee catchments and the Drumtee tributaries are apparent (see discussion in Chapter 4). Thus indirectly land use change may alter the Fe export via the effects on DOC concentration.

Between the Whitelee catchments, WL15 experienced recent deforestation due to the windfarm extension activity during 2010 – 2013 (Phin, 2015). The higher [DOC] in WL15 than other catchments occurred after extension started and was maintained during the sampling in this research. Thus, in addition to the largest peat cover, wind farm-associated [DOC] increase in WL15 may partially contribute to the larger [Fe] than others. Specifically, deforestation may increase more [DOC] than wind farm construction (see discussion in Chapter 4), and thus enhance larger Fe export in the draining waters (Fig. 5-1 and Fig. 5-2). A similar increased [Fe] after deforestation has also been reported elsewhere, e.g. in mid-Wales [Fe^{3+}] in upland waters increased over the past 20 years whose catchments had gradual/phased felling and clear felling, and total [Fe] increased within the soil following felling (Neal et al., 2008). Apart from changing DOM export, hydroecological water use and run-off patterns, felling operations can also cause disturbance and partial mineralization of top soil which then releases Fe into soil and pore water, contributing to an increased Fe concentration (Muller et al. 2015).

Water retention time: Further, DOC binding ability with Fe (indicated by Fe/DOC) also differed between the Whitelee catchments, and was positively related with HS proportion. Spatial variations of DOC composition were exhibited in Whitelee. With the samples being collected at the outlets of catchments with different areas, this allows the consideration of potential influence of water retention time on DOC composition changes. DOC decomposition rate was suggested to reduce with increasing water retention time (Catalán et al., 2016), and thus more humic and resistant DOC composition would be expected. In Whitelee, the longer flow length and thus water retention time in WL1 and WL16 (11.4 and 10.8 km, Chapter 2 Table 2-1) provided more chance for labile DOC decomposition, and likely contributed to the larger proportion of HS (indicated by the smaller E_2/E_4 in WL16 and WL1, Chapter 4 Fig. 4-7) than other catchments which all had flow length ranged from 5.3 – 8.1 km. As a result, greater DOC binding ability in WL16 and WL1 may be favoured by the greater HS proportion.

However, although WL14 flow length was longer than WL13 and 15, Fe/DOC in WL14 was clearly different from and constantly smaller than those in other catchments (Fig. 5-8). WL14 E_2/E_4 was not significantly larger than others, however an often smaller and more stable E_4/E_6 was shown in WL14 (Chapter 4 Fig. 4-7), indicating in the pool of HS, there

may be a greater proportion of large molecular weight HAs, contributing to the chance of DOC-Fe to aggregate (Kritzberg et al., 2014). Although WL14 flow length was not the longest, it is noticed there was a reservoir upstream in WL14 catchment (Chapter 2 Fig. 2-1) where more labile and FAs may be degraded, resulting in the greater HAs proportion in the carbon pool.

The understanding of potential impacts of different catchment characters on [Fe] and Fe/DOC molar ratio has important implications to water supply and land use management in peat-covered catchments. In this study, the deforestation-associated [Fe] increase, and the greater possibility of C precipitation in WL16 due to longer flow length should be taken into consideration when modelling catchment behaviour, for example if water treatment in these catchment is required.

5.5.3 Interaction between Fe and nutrients

Considering Fe interaction with nutrient dynamics may also reveal key biogeochemical process, with iron considered as a predictor of nutrient mobilization in some environments (Emsens et al., 2016). A weak and negative relationship (R^2 = 23 %) was observed between Fe and TON in the Whitelee catchments, which may arise due to the oxidation of Fe^{2+} via reduction of NO_3^- to NH_4^+ (Weber et al., 2006). Thus, when more Fe^{2+} were oxidised to Fe^{3+} and formed complexations with DOC contributing to high dissolved [Fe], more NO_3^- may be reduced, producing a gas not measured in [TON].

A relatively stronger and positive relationship was shown between Fe and SRP in peat-rich catchments, in agreement with other aquatic systems. Iron may enhance phosphorus complexation with DOC and thus facilitate total phosphorus export in catchment fluxes (Dillon and Molot, 1997), Experimentally it was observed that OM-Fe complexes can sufficiently suppress the formation of solid $FePO_4$ to result in increased dissolved P concentrations (Sundman et al., 2016), and so increased Fe may result in increased P export and bioavailability. Here, the complexation of Fe with DOC is suggested to contribute to high total dissolved [Fe] in the aerobic stream surface water and it may be this complexation facilitates great P export: when there were more DOC-Fe complexes, both soluble [SRP] and [Fe] increased as less interactions between Fe^{3+} and PO_4^{3-}. 32 % of variation in [SRP] may be explained by this organic complexation-related [Fe] (Fig. 5-10), and this DOC-mediated link between P and Fe may also explain some of co-varying [DOC] and [SRP] observed in this research (Chapter 4) and other studies.

However the changes in concentration increase/decrease were not the same for Fe and P, resulting in variable Fe/P ranging from 6 – 60 in the Whitelee catchments (Fig. 5-12). Fe/P in Whitelee is smaller than that in the ditch water of a catchment characterized by sandy soils with Fe-rich groundwater, which ranged from 16 – 1010 (Baken et al., 2015). However, it is larger than some other aquatic systems, e.g. Fe/P ranged from around 1 – 6 for Swedish lake fresh water and seawater samples (Gunnars et al., 2002). This may be mainly caused by organic complexation-associated higher [Fe] and typically lower nutrient concentrations in peat soils than those in lake water and seawater. In addition, the absorption of P to residual Fe (hydro)oxides was suggested as a mechanism for less efficient solubilisation of P than Fe (Baken et al., 2015). Thus in Whitelee, the higher Fe/P in summer may suggest a greater P sink via sorption to Fe oxides from land to fluvial systems. Similarly, the Fe/P molar ratio was used as a tool to estimate the P mobility in soil elsewhere. For example in a highly-degraded peat soil, high P exports would be expected when Fe/P in extractions of sediment was higher than 10 (Zak et al., 2010).

Taken all together, the increased [Fe] in Whitelee waters may contribute to the decrease in TON but increase in SRP availability, although in summer a greater P sink would be possible during export from land. These changes may further influence N and P and ultimately C biogeochemical cycles in fluvial networks.

5.6 Conclusion

The [Fe] in the Whitelee organic soil rich catchments was at the higher end of concentrations found in many peaty aquatic environments, but lower than that observed in soil pore water and groundwater. The similar seasonal pattern of [Fe] to [DOC], and the statistically significant covariance indicates a close interaction between Fe and DOC in the oxic surface waters. This is not surprising as the formation of stable complexes with DOC can help to maintain Fe^{3+} against aggregation and enable the transport of dissolved Fe^{3+}, and so coupled changes observed in Fe and DOC concentration are also likely, and observed here. Further Fe and DOC interactions were explored, that is, larger HS proportion can facilitate Fe export, while high [Fe] may however limit DOC export via co-precipitation.

Spatial variation in [Fe] was apparent among Whitelee long-term monitoring sites, which may be attributed to three reasons: peat soil cover, land use difference and water retention time. Large peat cover and wind farm development (particularly deforestation) can both favour Fe export by producing strong organic ligands (HS) for Fe-DOC complexes (e.g. greater [Fe] in WL15). In addition, water retention time may also play an important

role affecting spatial variation of [Fe], by changing FAs:HAs in the DOC pool. The significant and positive relationship with E_4/E_6 and Fe/DOC suggests FAs may be more favoured than HAs by Fe for the binding. This helps to explain the smallest Fe/DOC in WL14.

Additionally, the formation of DOC-Fe, may be sufficient to suppress $FePO_4(s)$ to some extent and increase SRP export. The significant positive correlation between [Fe] and [SRP] may indicate this mechanism is important in the Whitelee catchments: the high concentration and humic composition of DOC in Whitelee may not only increase dissolved and mobilised [Fe], but facilitate the availability of P. [TON] was negatively related to [Fe], and the Fe^{2+}-Fe^{3+} reduction/oxidation may be involved in N cycling in peatland.

Taken all together, these findings can help to better understand the potential but important role of Fe in C, P and N biogeochemical cycles in aquatic systems, which will have further influence on DOC decomposition in peatland catchments. The records of spatial variation in Whitelee [Fe] partially due to deforestation, and the greater DOC binding ability to Fe in dry periods are helpful for peatland and water quality management in the future. The understanding of interactions generated also offers some insight into thinking about the organic carbon loss in fluvial systems (e.g. DOC removal by co-precipitation with Fe oxides), particularly considering the peatland is a principal DOC source to the fluvial environment (Worrall et al. 2006).

6 Biodegradation of compositionally-different DOC from Drumtee

This chapter presents results from four batch incubations of stream water samples. The last incubation experiment was kindly carried out by Pauline Weihmann, an internship from the University of Strasbourg. However, she has not contributed to the interpretation of this data nor has seen this chapter. The data was integrated with the other three incubation results to consider the wider seasonality in DOC biodegradation from Drumtee.

6.1 Abstract

The breakdown of fluvial DOM from a peaty catchment is examined by conducting biodegradation incubations, to understand if there are seasonal and spatial differences in the biodegradability of DOM. Four 21-day stream water dark batch incubation experiments were carried out, with stream water collected in autumn (September 2015), early winter (November 2015), late winter (January 2016) and summer (May 2016). Stream samples from the Drumtee tributaries and the confluence were incubated. The results showed, compared to the findings from other aquatic environments, biodegradation of Drumtee water DOC was smaller: BDOC: from 0.60 – 2.74 mg l^{-1} C; BDOC %: from 2.9 – 12.1 %. This may be attributed to the shorter length of the incubation. However, the Drumtee BDOC loss was comparable with others when normalised for the length of incubation. Thus other factors are different measures of BDOC estimation and DOC compositional characteristics.

Weekly measurements of DOC composition and changes in nutrient concentration allowed the fluvial DOC biodegradation dynamic to be explored. Both UV-visible measurements and fluorescence excitation-emission matrices with parallel factor analysis (PARAFAC) were used to identify different carbon groups in DOC that were being degraded. Protein-like PARAFAC component 6 (C6) and TON showed clear responses to BDOC degradation, suggesting C6 may be an important BDOC pool with a likely close metabolic interaction with TON.

The analysis of compositional influence on fluvial DOC biodegradation showed that initial nutrient concentrations, SUVAs and C6 (and its relative contribution) were good as single predictors for BDOC (or percent BDOC). A multiple linear regression model was built to estimate BDOC (and percent BDOC), which further confirmed the importance of these characteristics as significant in an interaction. SUVAs and C6 have likely come out because they reflect the refractory/labile DOC pools, while SRP and TON may exert the influence

on BDOC by supporting bacteria growth or providing materials for the labile component to form.

It appears there was seasonality in Drumtee in-stream BDOC loss, with more DOC loss in late winter and summer, and less in autumn and early winter. Further, spatial variation in BDOC loss was also observed. In autumn and early winter more BDOC was lost in D-FF than D-WF, but BDOC % was similar. In late winter and summer more BDOC (and BDOC %) loss occurred in D-WF. This may be linked to the relative changes in nutrient concentrations and DOC composition between sampling and between sites. In January and May, the greater nutrient concentrations, smaller DOC aromaticity and more labile DOM materials in D-WF likely support more DOC biodegradation (both BDOC and BDOC %).

6.2 Introduction

Part of the DOC pool consists of small molecular weight compounds that can be directly transported across microbial cell membranes (Battin et al., 2008) and thus in both terrestrial and aquatic ecosystems, carbon mineralization of dissolved material occurs. DOC is a potential source of energy to aquatic organisms and can be consumed either in the water column (Bernhardt and Mcdowell, 2008) or in stream beds or the hyporheic zone (Fiebig et al., 1991). Therefore biodegradable DOC (BDOC) is a key regulator of ecosystem metabolism (Vonk et al., 2015). However the breakdown of DOC also influences the wider fluvial C cycle, by producing DIC, which can be degassed to the atmosphere as CO_2 (e.g. Long et al., 2015). Small streams can be major sources of CO_2 to the atmosphere (Fasching et al., 2014), however it is not yet well understood to what extent this is fuelled by the biological decomposition of terrestrially-released carbon (Fasching et al., 2014; Stutter et al., 2013).

The bioavailability of DOM is closely related to its origins and microbial processing but is not reflected in DOC concentrations (Chapelle et al., 2009; Shen et al., 2015). There is considerable variation in the extent to which DOC can be biodegraded, varying across environments and substrates. For example, during a 7-week incubation of DOM in precipitation water percolated through various forest litters, DOM from deciduous leaf litter was more biodegradable (up to about 75 %) while that from spruce needles was scarcely degraded (Hongve et al., 2000). In contrast, during a longer incubation (70 days) but of DOM from different soils, less proportion of DOM (5 – 39 %) was degraded, with the smallest utilisation of biodegradable DOM from soils from a raised peat bog and the largest from the blanket peat (Bowen et al., 2009). A similar range of soil DOM biodegradation (7 – 38 %) was observed with DOM collected along a soil-stream continuum of bog, forest

wetland and upland forest (Fellman et al., 2009). Furthermore, the biodegradation in DOM from soil was significantly greater compared to from stream water in the bog and forested wetland.

This soil composition control has been observed elsewhere. For example, more C was biodegraded (146 – 309 μg C l^{-1} day^{-1}) in storm flow runoff from a peat mire than from a forest (30 – 151 μg C l^{-1} day^{-1}) (Berggren et al., 2009). Further DOM from waters percolating through different soil profiles showed varying decomposition rates (Qualls & Haines, 1992). The biodegradability of DOM declined vertically with profile depth, with highest rates (48.7 %) in the throughfall and lowest in the soil A horizon (highly-decayed organic matter in mineral matrix, 13.9 % decomposed). However the biodegradability then increased with depth from the soil A horizon into mineral soil (26.1 %).

Variation in BDOC occurs across different aquatic systems: 11 – 27 % for stream DOC, 19 % for rivers and seawater DOC, and 14 % for lake DOC (Qualls and Haines, 1992; Søndergaard and Middelboe, 1995; Stutter et al., 2013; Volk et al., 1997). Groundwater appears low in BDOC (8 %) compared to other aquatic systems (Shen et al. 2015) and BDOC in coastal estuaries is more variable (8 – 29 %) (Moran and Hodson 1999; Raymond and Bauer 2000; Lønborg et al. 2009; Lønborg and Søndergaard 2009). Big rivers and lakes, which have longer water retention time, tend to have lower biodegradation rates (Catalán et al., 2016). The DOC will be more refractory having been in the fluvial network for a longer time (Moody, 2016). Furthermore, the extent of BDOC processing within one system can vary e.g., the fraction of BDOC in DOM decreased downstream the fluvial network in continuous permafrost regions, suggesting highly biodegradable DOC is lost in headwater streams (Vonk et al. 2015).

There are many environmental factors that can affect BDOC presence and differences in terrestrial and aquatic systems (Palmer et al., 2016). Regional characteristics including climate, surface geology and lake morphometry, regulate the lake removal of terrestrial DOC (Olefeldt et al. 2013), such that any environmental change that destabilizes the terrestrial C source has consequential impacts on in-stream C quality and hence shapes DOC biodegradability (Billett et al., 2006). Hydrological connectivity appears influential e.g. more DOC is biodegraded immediately after precipitation events and low biodegradation occurred in drier periods, suggesting high DOC biodegradability is supported by active recharge to the aquifer (Shen et al. 2015). Meanwhile DOM biodegradation is also strongly dependent on availability of nutrients such as nitrate and phosphorus. Amino acids, together with other low molecular weight, labile compounds (such as carbohydrates and carboxylic acids), are increasingly recognised as important

drivers of bacterial productivity in surface waters (Berggren et al., 2010; Stutter et al., 2013). Fresh plant-derived C and ammonium concentrations are found to be strongly and positively correlated with biological removal of DOC (Mann et al., 2012). In aquatic systems where nutrient levels are normally low, for example, peatland drainage, the biological processing may be slow relative to in-stream residence times (Palmer et al., 2016). Phosphorus has been described to play a central role in regulating the balance between catabolism and anabolism in aquatic systems like lakes, with great dissolved P shifting the balance to anabolism (Guillemette and Giorgio, 2012). Therefore phosphorus can accelerate the bacterial growth rate and efficiency (Smith and Prairie, 2004), which can lead to more BDOC degradation due to larger size of bacterial community.

Significant improvements in DOM characterization techniques have led to the understanding that DOM is a complex mixture of soluble organic compounds that vary in their reactivity and biochemical composition (Fellman et al., 2010), and more and more studies have proved a metabolic link between DOC composition and biodegradability (Fellman et al., 2010; Mann et al., 2012). UV-visible absorbance and fluorescence, commonly used to analyse DOM composition (as described earlier in Chapter 1, Section 1.7), can help understand processes of degradation. For example, in dark incubations of peatland porewater and lake water samples, there were weak but significant negative relationship between sample $SUVA_{254}$ and DOC loss during dark incubations (Olefeldt et al. 2013), indicating more biodegradation occurred with less proportion of aromatic DOC.

However, $SUVA_{254}$ may not alone significantly explain the BDOC variance, suggesting several factors may interact regulating DOC biodecomposition. For example, in the incubation of UK moorland DOC, $SUVA_{254}$, the sum of rainfall and $\delta^{15}N$ value of the DOM isolate, can together significantly describe BDOC ($R^2 = 0.66$, $p = 0.03$) (Stutter et al. 2013). Similarly, changes in DOM fluorescent characteristics have been suggested to reveal various BDOC changes in different systems. Humification index (HIX) (Fasching et al., 2014; Gabor et al., 2015), DOM freshness (β/α) (Fasching et al., 2014; Schiller et al., 2015) and the fluorescence index (FI) (Mann et al., 2012; Mcknight et al., 2001) derived from fluorescence are often used as a proxy for the extent of DOC humification, DOC freshness and source (i.e., terrigenous or microbially produced) respectively. For example, the use of HIX indicated a higher degree of humification as one of characteristics for terrigenous DOM delivery to various Austrian streams (than the DOM being autochthonous), and the decrease of HIX with a concurrent increase of β/α revealed that microbial degradation led to the changes of the optical properties of DOM and an enhanced autochthonous DOM signature (Fasching et al., 2014).

Additionally parallel factors analysis (PARAFAC) modelling of EEMs spectroscopy has been widely used in DOC biodegradation research to characterize different C pools. PARAFAC identified components have been linked to humic-like, fulvic-like and protein-like (including tyrosine- and tryptophan-like) fluorophores (as discussed in Chapter 1 Section 1.7), and suggested to be good predictors of BDOC. For example, it was revealed in Alaskan peatland soil water samples, the presence of protein-like fluorescence was significantly and positively correlated ($p < 0.001$; $R^2 = 0.82$) with BDOC turnover for soil waters taken together from bog, forested wetland, fen and upland forest (Fellman et al., 2008), which is consistent with laboratory results showing amino acids to be a readily available source of C, N and energy for heterotrophic microbes (Ellis et al., 1999).

Given the observed differences in DOM composition within the Drumtee catchments (chapter 4), to understand how in-stream respiration may contribute to the DIC pool (and so to the capacity for the river to degas CO_2 to the atmosphere) and how this may vary seasonally, seasonal batch incubations of DOM from the two tributaries and the confluence in the Drumtee catchment to assess the rate of BDOC uptake were undertaken. Measurements of UV-visible and fluorescence composition, and nutrient concentration were undertaken to characterise the DOM pool and how this changed as BDOC was mineralised. The specific aims of this research were to:

1. Quantify the BDOC component in Drumtee stream water from different seasons and sites, using 21 – day incubation, and examine if there is spatial and/or temporal variation in BDOC contents.

2. Characterize fluvial DOC in samples from Drumtee stream water during its biodegradation, using UV and fluorescence spectroscopy, and assess if there are changes in the composition of DOC, and nutrient (N and P) concentration.

3. Explore the relationship between fluvial BDOC loss, and nutrient availability and spectrophotometric composition in this peaty catchment to establish how these influence BDOC.

6.3 Materials and methods

6.3.1 Sampling and incubation strategy

Water samples were collected from Drumtee catchment using five litre PETE drinking water bottles on 07/09/2015, 17/11/2015, 19/01/2016 and 31/05/2016. These dates were chosen to span a seasonal range: autumn, early winter, late winter (surface frozen),

and summer respectively. The bottles were pre-cleaned before sample collection using the same protocol described in the method chapter.

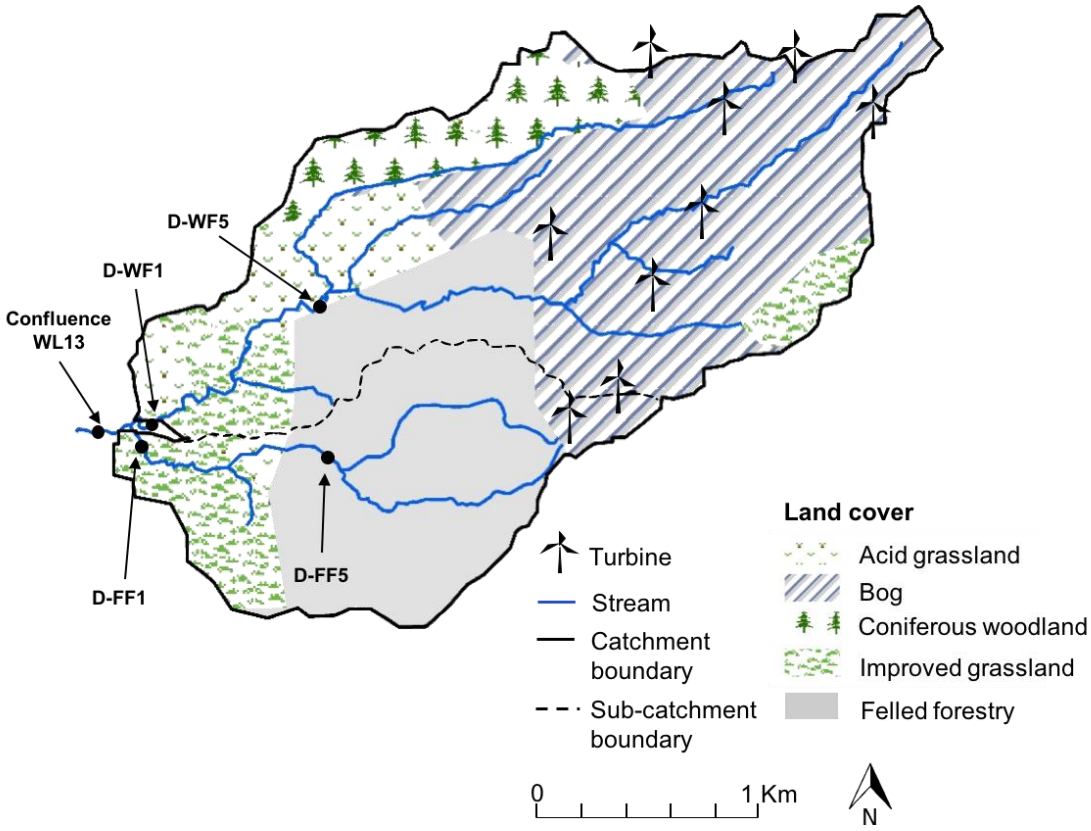


Fig. 6-1. Drumtee catchment and sampling sites (indicated by black dots) in the sub-catchments D-WF and D-FF, and the confluence WL13. Land use data was from Digimap® Land Cover Map 2015.

In the September incubation, water samples were collected from two sites in Stream D-WF (D-WF1, 5), two sites in Stream D-FF (D-FF1, 5) and one from the confluence WL13. However due to logistical reasons it was difficult to manage all sample analysis during short incubation sampling interval, thereafter only one site for each stream (D-WF1, D-FF1) and the confluence, WL13, were sampled for incubation in Nov. 2015, Jan. 2016 and May 2016 respectively (Fig. 6-1). Due to the lack of temporal comparison, the data for D-WF5 and D-FF5 are not discussed in the following sections.

Water samples were returned to the lab within four hours and stirred overnight in temperature controlled (20 °C) dark room to expel oxygen in excess of atmospheric equilibrium. As the result of method development, a large amount of small reagent bottles were used as sealed individual incubation systems (Chapter 3, Section 3.2). Therefore on the next day, the well-mixed five litre sample was dispensed into 90 brown, 250 ml, air-tight glass reagent bottles, with no headspace, except for September when 60 bottles were filled. This number of sub-samples allowed five replicates per site in the

November, January and May incubations, and two replicates per site in the September incubation to be sacrificially sampled six times. In order to consider the net fate of DOC and include production from and absorption by POC, which can happen in streams (Moody et al., 2013), samples were not filtered before incubation. No nutrient supplement was provided (see discussion in Chapter 3, Section 3.2). Once in the reagent bottles, samples were incubated for three weeks in the dark at 20 °C, without further treatment.

6.3.2 BDOC loss analysis

Changes in [DO] were chosen to quantify BDOC degradation (Chapter 3, Section 3.2). Using a DO meter (HQ10 Hach Portable LDO™), [DO] was measured from the 250 ml sub-samples every 3 or 4 days (Table 6-1). Collected samples had been kept in the temperature-controlled incubation room before the incubation started, so they were allowed to adjust to room temperature (also ~ 20 °C) when being measured. Seven replicate readings were taken for each sample when the meter was stable, and the mean values were taken as representatives. The sub-sample was thereafter used for further chemical analysis, or discarded (Table 6-1).

The observed [DO] change was converted to BDOC loss using the equation: degraded C (mg l^{-1}) = [DO]/32*12 (mg l^{-1}). However, the absolute BDOC loss variation may be influenced by availability of BDOC and so DOC starting concentration, thus to better compare the decomposition rate independent of [DOC], the BDOC % loss as a proportion of starting [DOC] was calculated. The preliminary trials showed the Thermalox™ TOC analyser was not precise enough to detect the small changes of sample [DOC] during incubations (Chapter 3). Therefore, only the initial [DOC] on Day 0 was measured for other relevant calculations of parameters.

Table 6-1 The lab work schedule during each incubation. [DO] was measured more frequently than the weekly DOC compositional analysis using UV-visible absorbance and fluorescence measurements, and nutrient measurements. [DOC] was only analysed at the beginning of incubations. Incubations were conducted in dark at 20 °C for 21 days.

Measurement	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21
[DO]	✓	✓	✓	✓	✓	✓	✓
[DOC]	✓						
[TON]	✓		✓		✓		✓
[SRP]	✓		✓		✓		✓
UV absorbance	✓		✓		✓		✓
Fluorescence	✓		✓		✓		✓

6.3.3 Water nutrient and DOC composition analysis

The 250 ml sample was used to characterise weekly sample chemical composition: [SRP], [TON], spectrophotometric UV and fluorescence EEM measurements (Table 6-1). PARAFAC fluorescence model was further applied to examine the complete EEM dataset and identify fluorophores of incubation DOC. All the sample preparation and analysis were undertaken as described in Chapters 2 and 3.

6.3.4 Data analysis

One-way ANOVA was carried out in Minitab 17 using Tukey pairwise comparisons, to identify if a significant difference of mean values occurred among sites or dates ($p < 0.05$). Correlations were tested using Pearson correlation in Minitab 17, between BDOC (or BDOC %) loss and initial [TON], [SRP], and other parameters of sample composition as characterised by, for example, UV-absorbance characteristics, fluorophore component intensity. Mean values of replicates for each site on Day 0 were used for analysis, and relationships were considered significant when $p < 0.05$. Simple linear regression was used to test variation explained by each of the parameters, and multiple linear regression model was explored to identify which characteristics co-explained the greatest variation in BDOC (or BDOC %) across sites and dates.

6.4 Results

6.4.1 Intra-sample variation of initial [DOC], [SRP] and [TON]

For all three sites sampled (confluence, D-FF1 and D-WF1), [DOC] ranged from 20.0 – 78.2 mg l^{-1} C, with all sites sharing the same seasonal pattern of the greatest [DOC] concentrations in summer/autumn (September 2015, 47.0 ± 22.2 mg l^{-1} C), and smallest in winter (January 2016, 22.3 ± 3.4 mg l^{-1} C) (Fig. 6-2A). D-FF1 always had higher [DOC] than D-WF1, and the confluence WL13 had a [DOC] intermediate to these sites. The [DOC] in spring and summer was more variable between D-WF1 and D-FF1, but became less so in the winter months.

[SRP] ranged from 15.8 ± 0.2 µg l^{-1} (D-WF1 in November) to 62.1 ± 2.2 µg l^{-1} (D-WF1 in January, Fig. 6-2B). The intra-sample variation in September and November was similar to initial [DOC], with [SRP] greatest in D-FF and smallest in D-WF. However, when [SRP] of D-FF decreased in January and May, D-WF and the confluence site WL13 did not share this trend but increased in concentration.

In September and November, [TON] was similar among sites during each month (although no TON was detectable in D-FF in September, limit of detection is 0.3 µg l^{-1}), with concentrations ranging from 11.9 ± 0.0 µg l^{-1} (D-WF1 in September) to 38.2 ± 1.7 µg l^{-1} (D-FF1 in November) (Fig. 6-2C). There was a clear increase in [TON] in January 2016 for all three sites, with the concentration ranging from 109.3 ± 2.0 µg l^{-1} (D-WF1) to 117.9 ± 1.8 µg l^{-1} (D-FF1). In May the D-WF1 and WL13 [TON] was greater at 158.3 ± 1.7 µg l^{-1} and 149.1 ± 15.1 µg l^{-1} respectively. However, a large decrease in [TON] was measured in D-FF1, with 24.9 ± 9.7 µg l^{-1}.

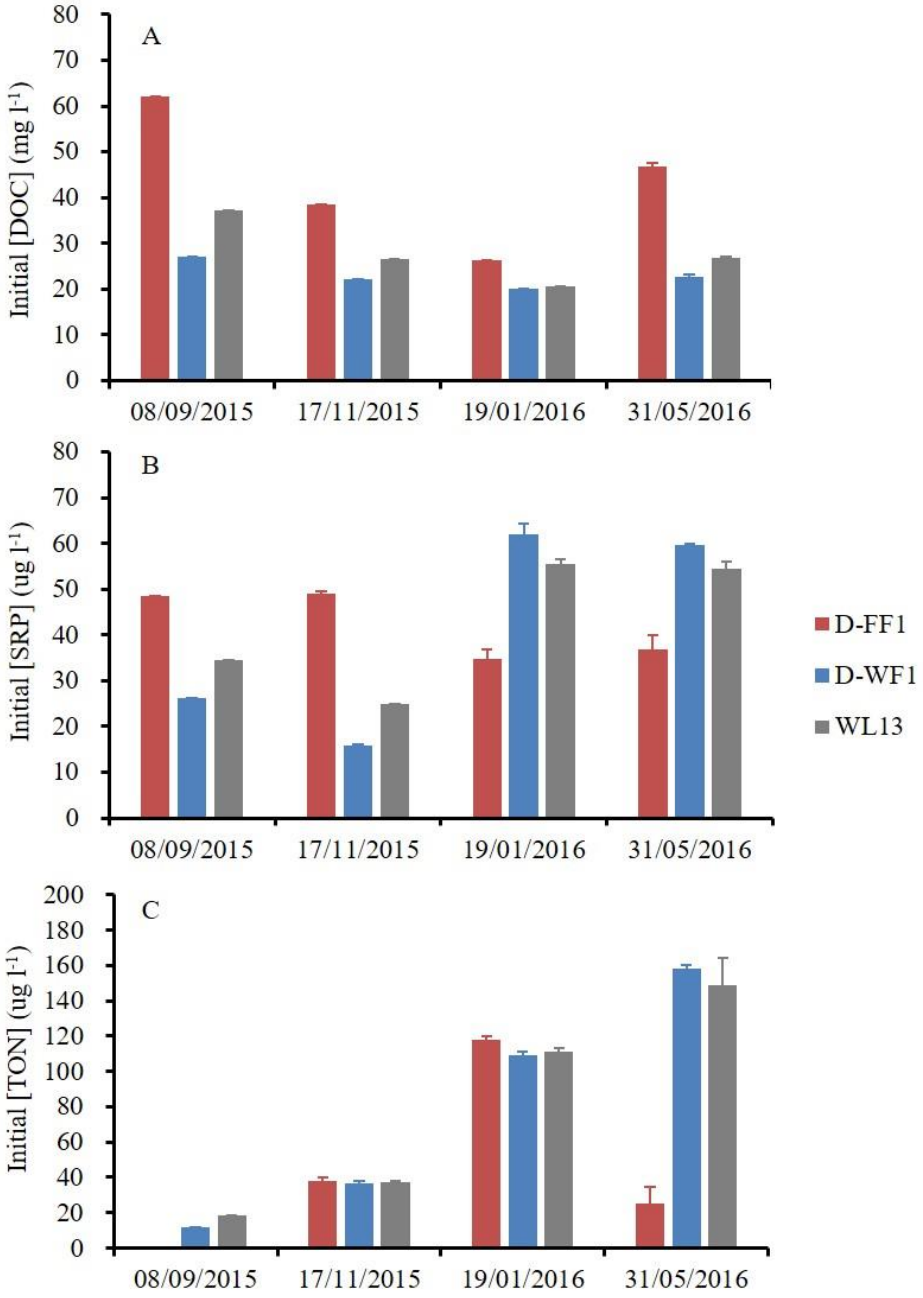


Fig. 6-2. The average concentrations of (A) DOC, (B) SRP and (C) TON measured at the beginning of the incubations. Nutrient concentrations of D-WF and WL13 were highest in January 2015 and May 2016. There was no detectable [TON] for September D-FF. Error bars (± 1SD) are showing the standard deviation among replicates in each site.

6.4.2 Total BDOC loss in 3-week incubation

The calculated loss in [DOC] across all incubations ranged from 0.63 ± 0.08 – 2.74 ± 0.03 mg l^{-1}. The largest loss was observed in the May incubation and smallest in November (Fig. 6-3A). Between all sites, in September and November DOC samples from D-FF1 showed the largest loss of BDOC (1.78 ± 0.00 and 1.12 ± 0.16 mg l^{-1} respectively), while in those months, D-WF1 incubations had the lowest BDOC loss (1.04 ± 0.13 and 0.63 ± 0.08 mg l^{-1} respectively). However in the January and May incubation, the largest BDOC losses were observed in D-WF1 (2.43 ± 0.03 and 2.74 ± 0.03 mg l^{-1} respectively) and the smallest losses in D-FF1 (1.22 ± 0.09 and 1.91 ± 0.18 mg l^{-1} respectively), which previously had the highest losses.

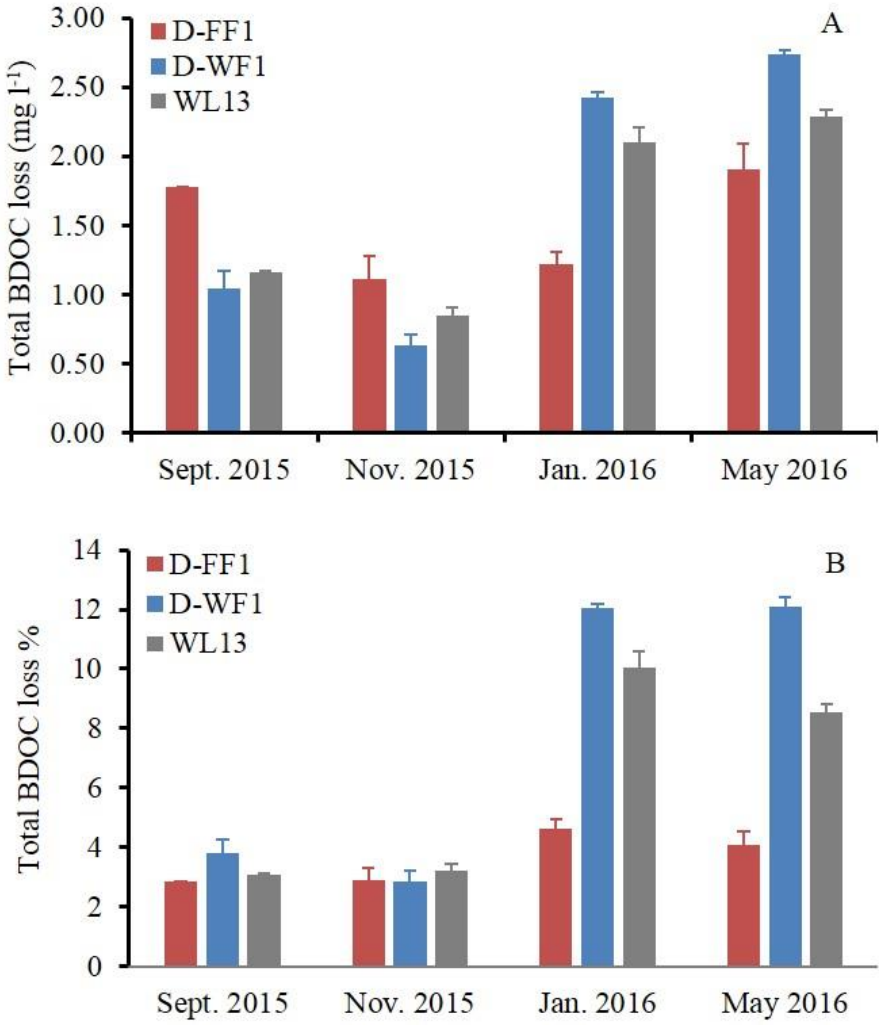


Fig. 6-3. The total loss of biodegraded DOC (BDOC) over the three-week incubation (A), and the percentage loss of the BDOC (B) as a function of initial DOC concentration. Error bars (± 1SD) indicates the standard deviation among replicates in each site.

The pattern is a little different when BDOC loss is considered as a function of starting concentration. BDOC loss (%) ranged from 2.9 – 12.1 % over the three-week incubation

(Fig. 6-3B). For all site taken together, BDOC % in the September (3.2 ± 0.5 %) and November (3.0 ± 0.2 %) incubations were very similar, with small inter-site variations. However in the January and May incubations a greater proportion of the BDOC was lost (8.9 ± 3.8 and 8.2 ± 4.0 % respectively) with more obvious inter-site differences. D-FF1 % DOC loss was the same in September and November, but slightly increased by 1.7 % in January, and decreased by 0.5 % in May. In January and May, the total % DOC loss was much great for D-WF1 (12.0 ± 0.17 and 12.1 ± 0.32 % respectively) and WL13 (10.1 ± 0.52 and 8.6 ± 0.25 % respectively) than for September and November (Fig. 6-3B). Although total D-FF1 BDOC loss was largest of all three incubations in September and November, the % DOC loss was consistently the smallest of all three sites.

6.4.3 UV character of initial samples

The initial $SUVA_{254}$ ranged from 2.9 ± 0.1 (D-WF1 in May) to 4.1 ± 0.0 l mg^{-1} m^{-1} (D-WF1 in November), and $SUVA_{410}$ from 0.41 ± 0.01 (D-WF1 in May) to 0.58 ± 0.00 l mg^{-1} m^{-1} (D-WF1 in November). Most of the values for both SUVAs were within the range across all Drumtee sites during 2014 – 2015 ($SUVA_{254}$: 2.8 ± 0.3 to 4.5 ± 0.3 l mg^{-1} m^{-1}; $SUVA_{410}$: 0.38 ± 0.04 to 0.56 ± 0.03 l $mg^{-1}m^{-1}$). There was little variation in initial D-FF1 $SUVA_{254}$ and $SUVA_{410}$ composition across incubations ($SUVA_{254}$: 3.7 ± 0.1 l$mg^{-1}m^{-1}$, $SUVA_{410}$: 0.49 ± 0.02 l$mg^{-1}m^{-1}$, Fig. 6-4, Table 6-2). In contrast, the SUVA composition of D-WF1 and WL13 did vary, to be greater in November than September in 2015, and then lower in January and May 2016, more so in D-WF1 ($SUVA_{254}$: down to 2.9 ± 0.1 l$mg^{-1}m^{-1}$, $SUVA_{410}$: down to 0.41 ± 0.01 l mg^{-1} m^{-1}).

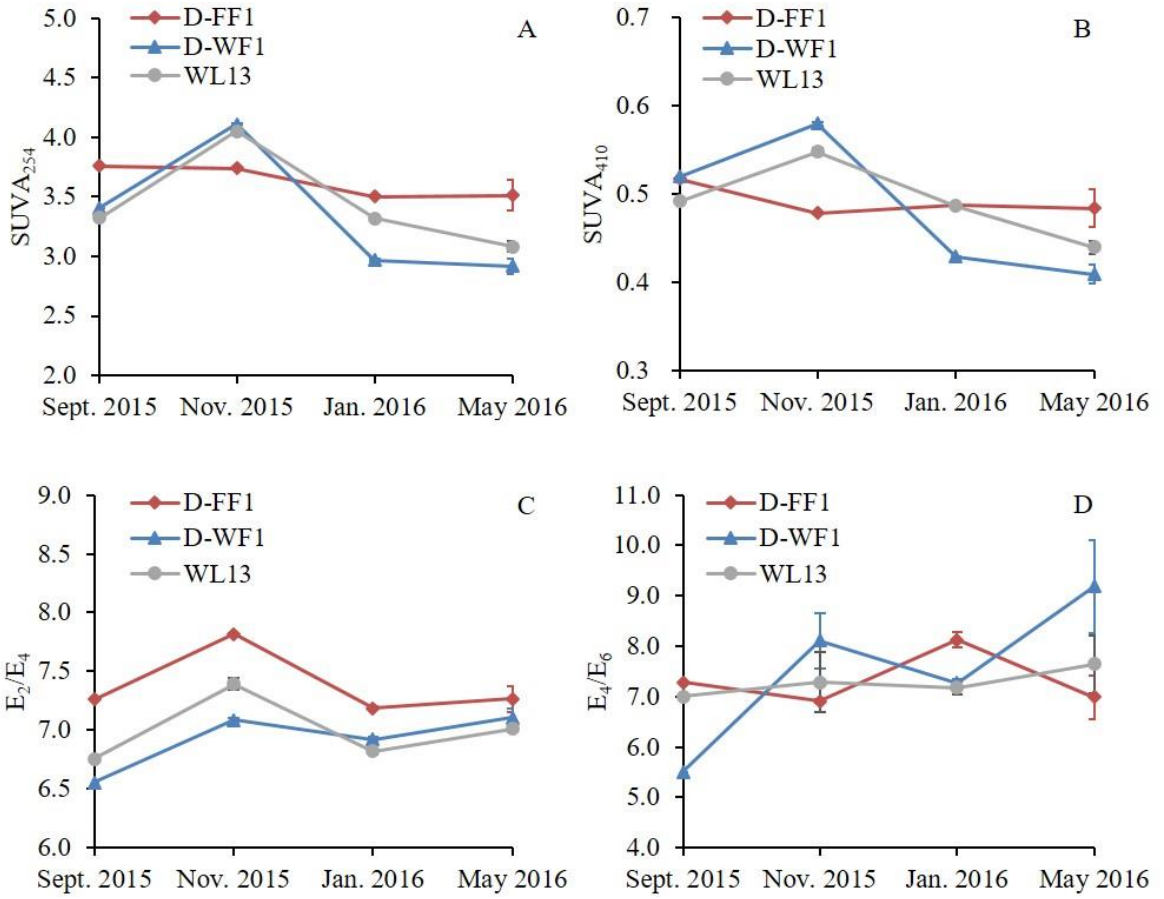


Fig. 6-4. The (A) $SUVA_{254}$, (B) $SUVA_{410}$, (C) E_2/E_4 and (D) E_4/E_6 values of each site starting composition for the three sampling months. Error bars indicates the standard deviation among the replicates in each site, and if not apparent they are smaller than the symbol.

E_2/E_4 of all sites showed more consistent temporal variation, with the values greater by about 0.5 units in November 2015 than September but lower in January 2016 to have a composition more similar to September 2015 (Fig. 6-4C). E_2/E_4 increased slightly in May 2016. E_2/E_4 had larger values in D-FF1 (7.4 ± 0.3) throughout the four incubations compared to the other two sites (D-WF1: 6.9 ± 0.3; WL13: 7.0 ± 0.3).

E_4/E_6 in D-FF1 (6.9 ± 0.03 – 8.1 ± 0.15) and D-WF1 (5.5 ± 0.0 – 9.2 ± 0.9) was variable in these four incubations, and they usually had opposite time series trend to each other (Fig. 6-4D). E_4/E_6 in D-WF1 may be increasing across the four sampling periods, but it is difficult to conclude this as the variation between sampling is unknown, and there were larger replicate variations in November and May. E_4/E_6 in WL13 was consistent over the four months (7.2 ± 0.1).

The values of both E ratios were within the range across all Drumtee sites during 2014 – 2015 (E_2E_4: 6.7 ± 0.5 to 9.5 ± 1.0; E_4/E_6: 5.3 ± 0.8 to 9.4 ± 0.6) except D-WF1 in September 2015 (6.5 ± 0.0).

Table 6-2 Initial [DOC] and total BDOC losses during 3-week incubation over the four sampling months are shown in the table. Initial [TON], [SRP], and optical parameter values for each site starting composition are also presented. The mean and standard deviation for each site for the four incubations are given. The initial [TON] of D-FF1 in September 2015 was below detection limit (0.3 ug l^{-1}).

Site	Month	[DOC] (mg l^{-1})	BDOC (mg l^{-1})	BDOC (%)	[TON] (ug l^{-1})	[SRP] (ug l^{-1})	$SUVA_{254}$ (l mg^{-1} m^{-1})	$SUVA_{410}$ (l mg^{-1} m^{-1})	E_2/E_4	E_4/E_6
D-FF1	Sept.	62.0	1.78	2.9	/	48.5	3.8	0.52	7.3	7.3
	Nov.	38.4	1.12	2.9	38.2	49.0	3.7	0.48	7.8	6.9
	Jan.	26.3	1.22	4.6	117.9	34.8	3.5	0.49	7.2	8.1
	May	46.9	1.91	4.1	24.9	36.8	3.5	0.48	7.3	7.0
	Mean	43.4	1.51	3.6	45.3	42.3	3.7	0.49	7.4	7.4
	SD	15.0	0.39	0.9	51.0	7.5	0.1	0.02	0.3	0.6
D-WF1	Sept.	27.1	1.04	3.8	11.9	26.1	3.4	0.52	6.6	5.5
	Nov.	22.0	0.63	2.9	36.3	15.8	4.1	0.58	7.1	8.1
	Jan.	20.0	2.43	12.0	109.3	62.1	3.0	0.43	6.9	7.3
	May	22.7	2.74	12.1	158.3	59.5	2.9	0.41	7.1	9.2
	Mean	23.0	1.71	7.7	78.9	40.9	3.5	0.51	6.9	7.0
	SD	3.0	1.03	5.0	67.2	23.4	0.6	0.08	0.3	1.3
WL13	Sept.	37.2	1.16	3.1	18.7	34.5	3.3	0.49	6.8	7.0
	Nov.	26.5	0.85	3.2	37.0	24.8	4.0	0.55	7.4	7.3
	Jan.	20.7	2.10	10.1	111.3	55.6	3.3	0.49	6.8	7.2
	May	26.7	2.29	8.6	149.1	54.6	3.1	0.44	7.0	7.6
	Mean	27.8	1.60	6.2	79.0	42.2	3.6	0.51	7.0	7.2
	SD	6.9	0.70	3.6	61.5	15.2	0.4	0.03	0.3	0.1

6.4.4 Fluorescence spectroscopic character of initial samples

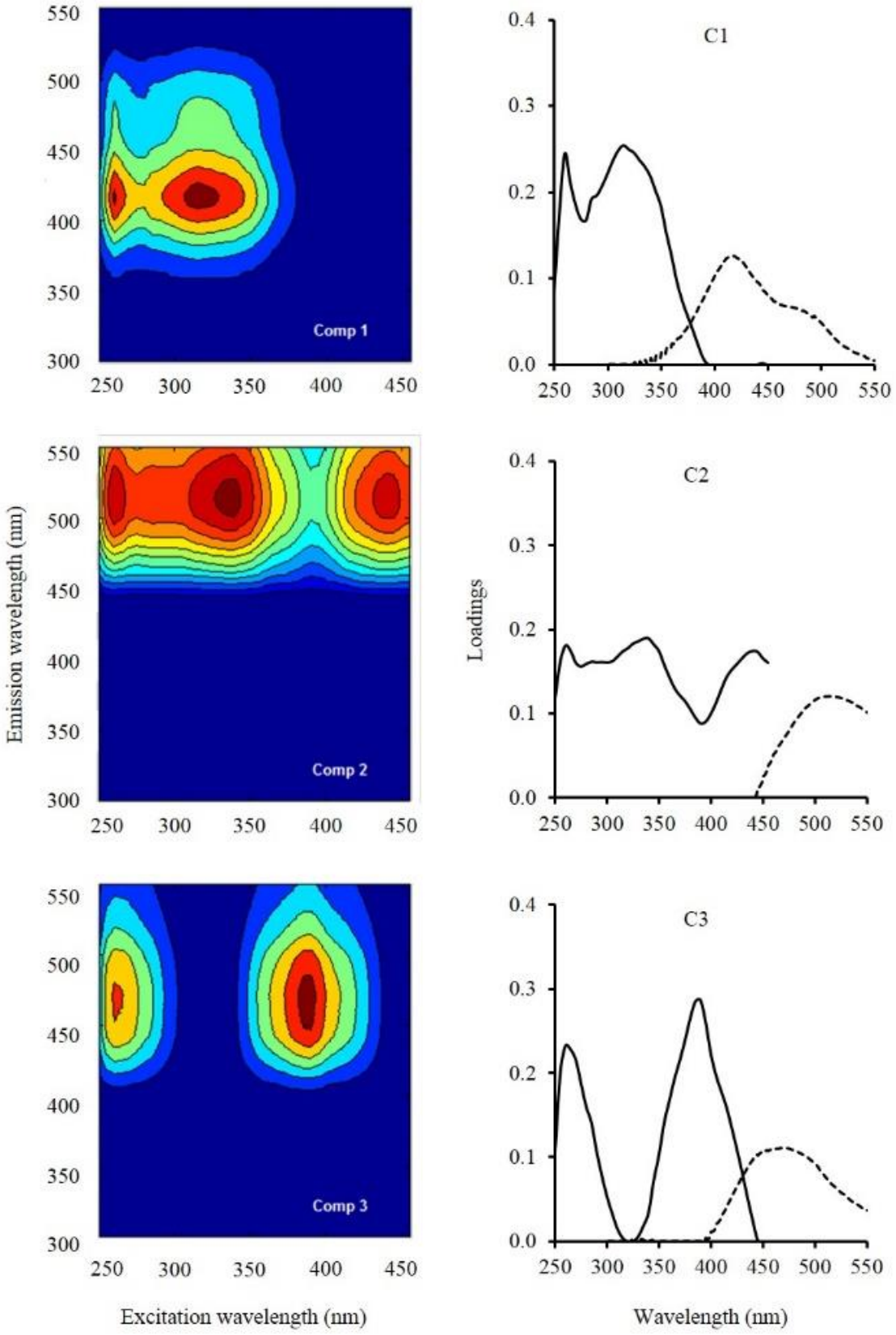
Only the six-component PARAFAC model was verified with split-half analysis. This means the six corresponding components in each sample had equal excitation and emission loadings as verified by the Tucker's congruence coefficients being greater than 0.95, between split halves, as well as between the complete dataset and a split half.

Table 6-3 The six components validated by half split analysis, with positions of each fluorescence maximum. The appearance of these components in other studies and their interpretation are listed. The component names in each reference column are the names identified in each referenced study.

Component	Excitation maxima (nm)	Emission maxima (nm)	Classic peak identification[1]	Description corresponding to previous studies
C1	315 (260)	418	A (humic-like); M (marine humic-like)	Humic-like, terrestrial[3, 5], common to a wide range of freshwater environments[3]
C2	340 (260, 440)	513	/	Fulvic acid-like, microbial-transformed[5], associated with higher plant origin[2]
C3	390 (260)	472	A, C (humic-like)	Humic-like, terrestrial[4, 7], recalcitrant and aromatic[7], biogeochemical processing of terrestrial POM[4]
C4	<260	427	A (humic-like)	Humic-like, terrestrial[3, 4], dominated DOM in warmer months[3].
C5	340 (260)	444	C (humic-like)	Humic-like, terrestrial/anthropogenic[3, 4, 6], exported from agricultural catchments[3], components of lignin and tannins[6]
C6	285	300 (350, 450)	T (tyrosine- and tryptophan-like)	Derived from autochthonous processes[2, 3], recent biological production[6], degradation may be important for dynamics[4]

1 (Coble, 1996); 2 (Cory & Mcknight 2005); 3 (Stedmon and Markager, 2005b); 4 (Yamashita et al., 2008); 5 (Williams et al., 2010); 6 (Osburn et al., 2012); 7 (Hosen et al., 2014)

The six components, C1 – C6 have all been previously observed by other researchers either using a PARAFAC modelling or classic visual EEMs peak picking (Table 6-3), and are presented in Fig. 6-5. Comparison with components identified previously (Table 6-3) suggests DOC in Drumtee water samples contains humic-like, fulvic-like and protein-like fluorophores. Humic-like fluorophores are mainly terrestrial-derived, with fulvic-like and protein-like ones from microbial processing. C1 consists of two major peaks, which have been ascribed to humic-like (peak A) and marine humic-like (peak M) respectively (Coble, 1996). C2 has been identified in research using PARAFAC modelling, and ascribed to microbial-transformed fulvic-acid like fluorophore (Williams et al., 2010). C3, C4 and C5 had humic-like fluorophores with single or multiple peaks, which were identified as peak A/C in classic peak identification. C6 is identified as protein-like, a mix of tyrosine-like (ex = 285 nm/em = 305 nm) and tryptophan-like (ex = 305 nm/em = 350 nm) fluorophores.


Comp 1
Comp 2
Comp 3
C1
C2
C3
Emission wavelength (nm)
Excitation wavelength (nm)
Loadings
Wavelength (nm)

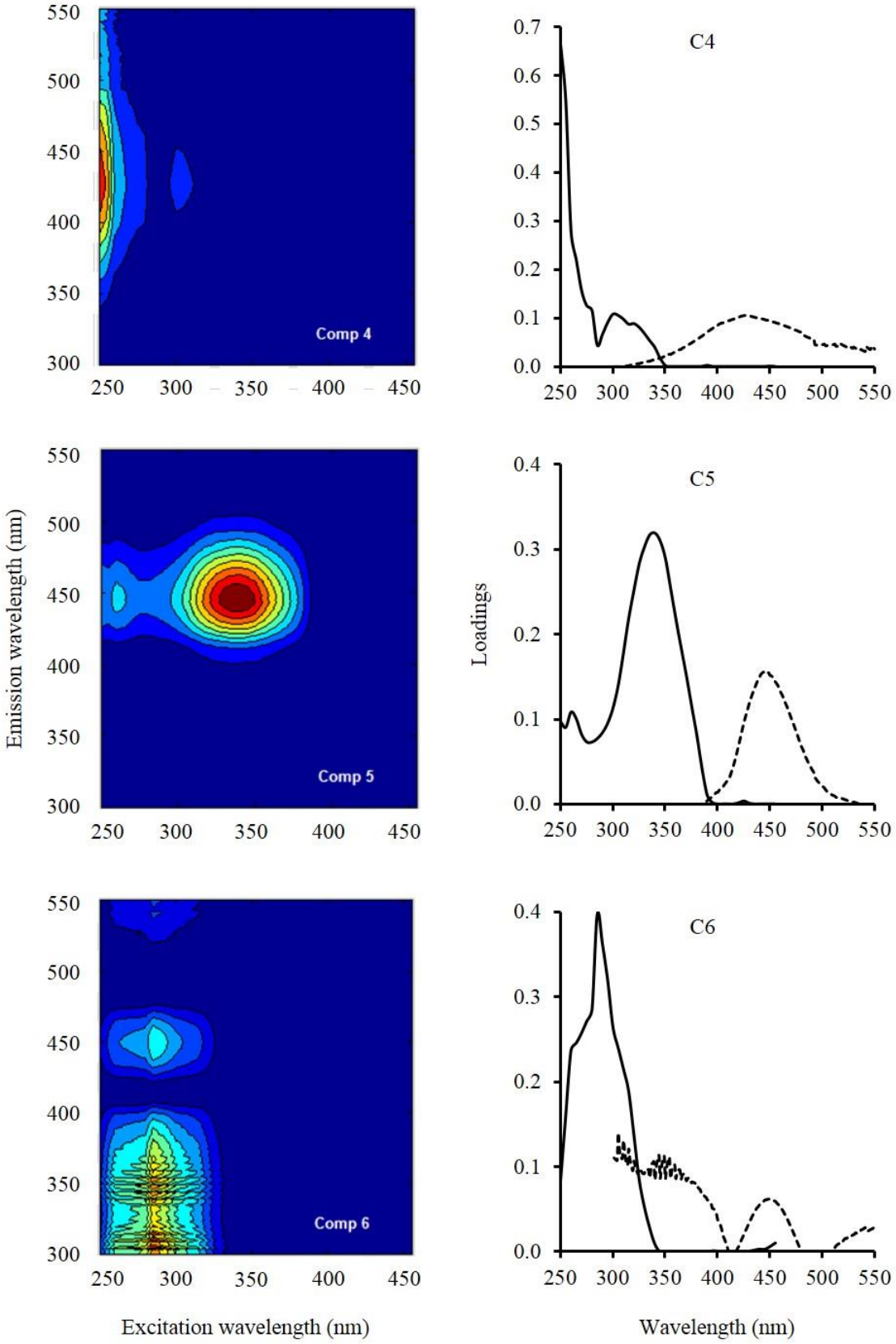


Fig. 6-5. Contour plots (left) of the six components C1 – 6 verified by the PARAFAC modelling with 203 incubation sub-samples. The line plots (right) show the loadings for the excitation (solid black) and emission (dotted black) in each component.

Fig. 6-6 shows the incubation starting spectrophotometric composition for the four seasons, represented by fluorescence intensity for PARAFAC-identified EEM fluorophore components (top), and contribution of each component to the total intensity (bottom). The different sampling locations showed a similar inter-season trend, with total DOM fluorescence intensity being consistently smaller in all sites in autumn to spring (0.43 ± 0.01 – 0.97 ± 0.03 R.U. across sites), and increasing in the summer (1.92 ± 0.01 – 4.95 ± 0.48 R.U.).

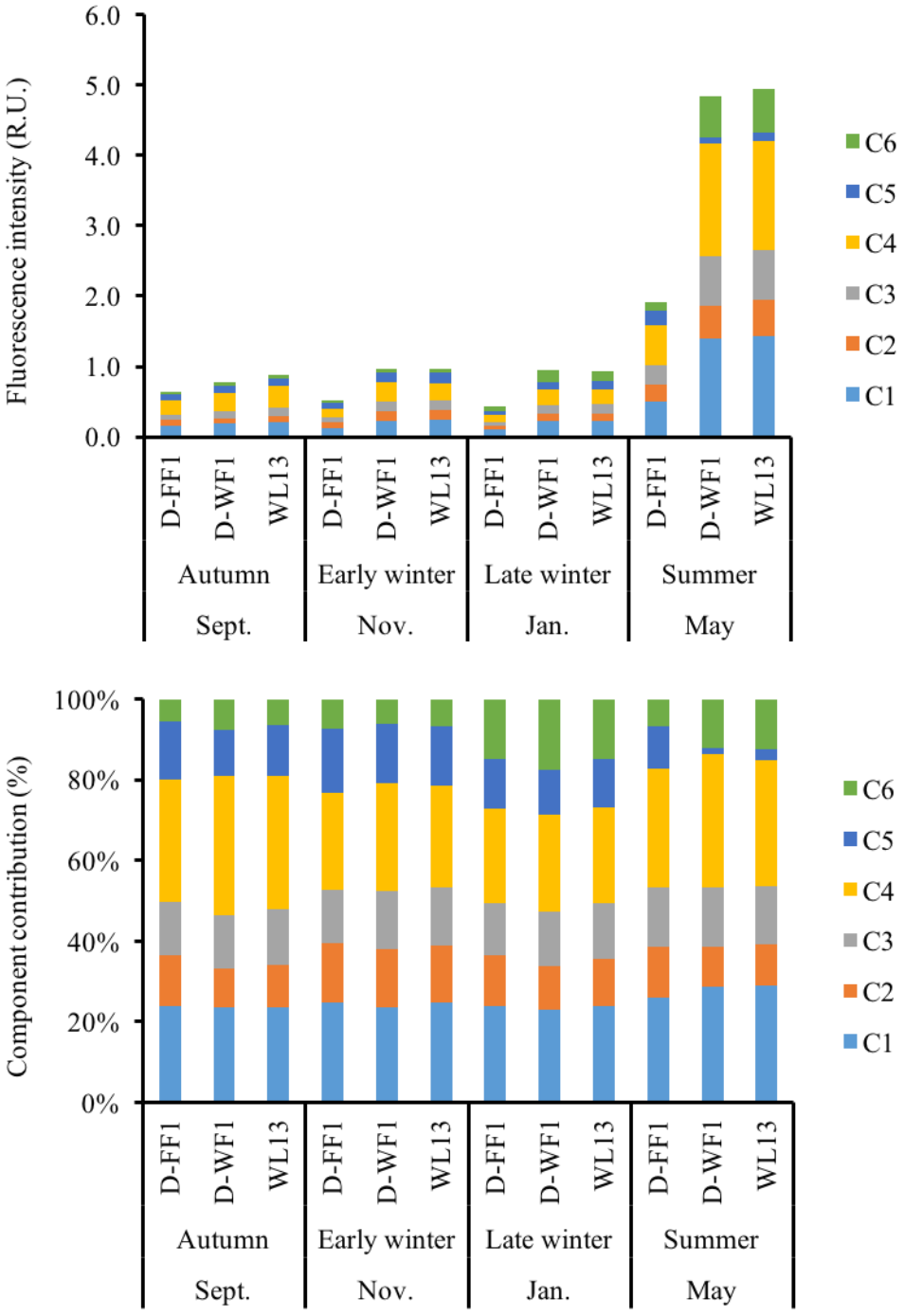


Fig. 6-6. The seasonal changes of the six PARAFAC component intensities (upper) and relative contribution (lower) for initial samples collected from September (autumn), November (early winter) 2015, January (late spring) and May (summer) 2016 across the three sites.

The spatial difference of total fluorescence intensity was clear with D-WF1 and WL13 samples having similar and larger amount of fluorescence intensity than D-FF ($p < 0.01$). This spatial difference was much more obvious in the summer, with the intensity of D-WF1 and WL13 (4.84 ± 0.18 and 4.95 ± 0.48 R.U. respectively) being more than twice that of D-FF1 (1.92 ± 0.01 R.U.). It seemed to be C1 (increased from 0.10 – 0.22 R.U. in spring to 0.50 – 1.43 R.U. in summer) and C4 (0.10 – 0.23 R.U. in winter to 0.56 – 1.60 R.U. in summer) that were increasing most. C5, showed least variation across seasons and sites, staying from 0.05 ± 0.00 (D-FF1 in spring) to 0.21 ± 0.00 R.U. (D-FF1 in summer). It is summer, the C6 intensity (produced from recent microbial process) of D-WF1 and WL13 was clearly greater than that of D-FF1.

The contribution of each PARAFAC component to total intensity was considered to allow comparison without dependent of [DOC]. C1 and C4 dominated the incubated DOM in these sites, comprising about 25 ± 2 % and 28 ± 4 % of the total intensities respectively for all data taken together. C2 and C3 were next in relative contribution, consistently comprising 12 ± 2 % and 14 ± 1% of the total intensities. C5 and C6 varied spatially and temporally in the percentage, and generally contributed the less than other components. While C1 – C4 remained similar in their contributions across changes in total intensity, this was not so with C5 and C6. C5 relative contribution decreased clearly in summer for D-WF1 and WL13 whereas remained similar for D-FF1. Relative contribution of C6 increased in spring and summer for all sites except D-FF1 in summer, which decreased to the similar percentage in autumn and winter.

6.4.5 Intra-incubation variation

6.4.5.1 BDOC loss

The respiration of BDOC for Drumtee did not occur linearly, but exhibited a trend more logarithmic in curve with an often-steeper slope in the first three days, a decrease in this slope between days to 14 and then the shallowest gradient in days 14 onwards (Fig. 6-7). This pattern is weak and not all responses were found in each incubation, but those that are can be summarised in Table 6-4. However, the logarithmic response apparent in considering BDOC loss in mg l^{-1} C sometimes disappeared when considering it as BDOC % loss. For example, D-FF1 % in all four incubations showed a more linear trend, whereas that in BDOC loss was more logarithmic, especially in September (autumn) and June (summer). BDOC % loss in D-WF1 and WL13 changed logarithmically only in January (late winter) and June (summer), while the corresponding BDOC loss was more in most months except November (early winter).

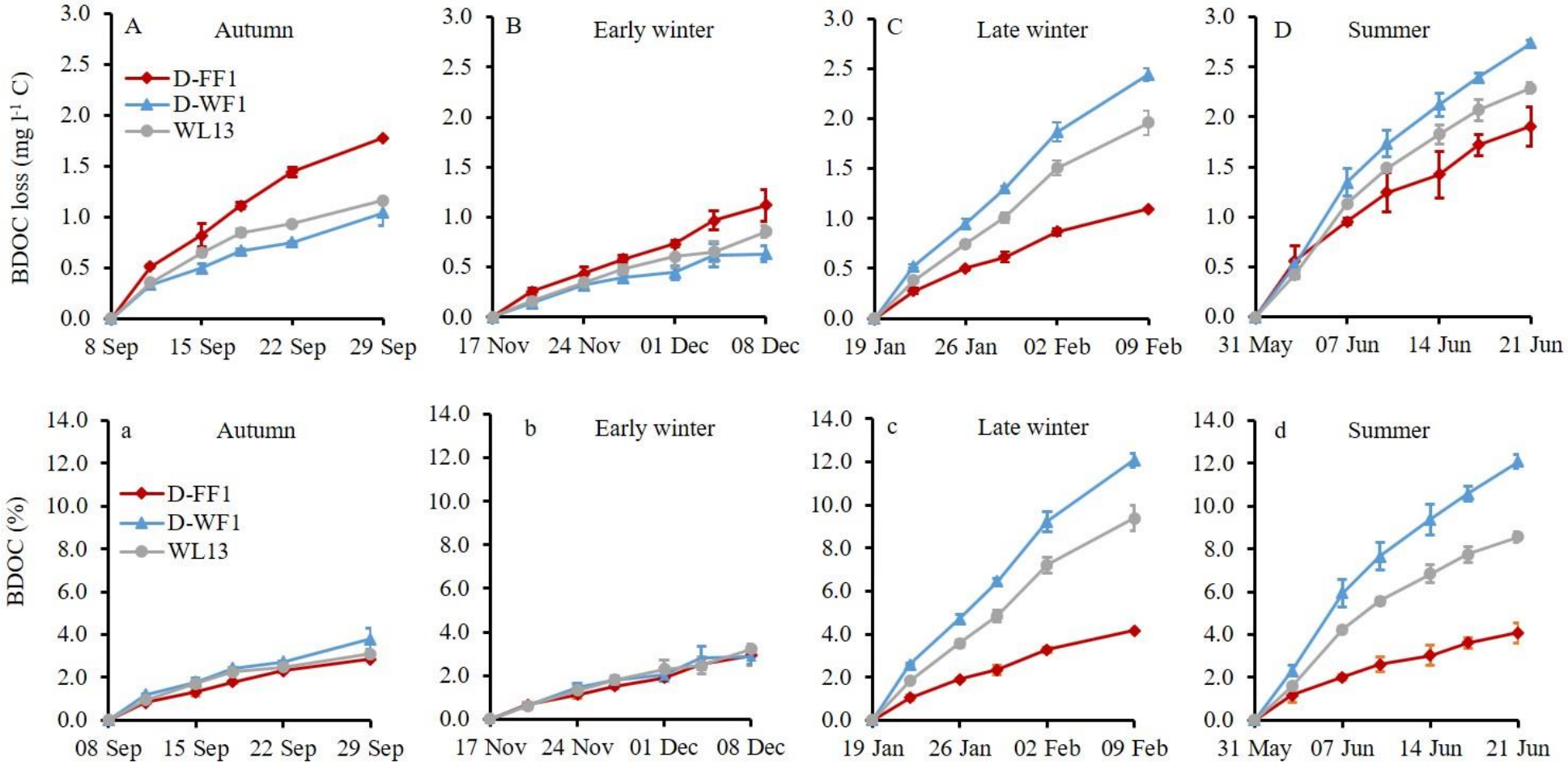


Fig. 6-7. BDOC loss (A – D) and BDOC (%) (a – d) changes during each incubation of samples from all sites in different months. The error bars (± 1SD) indicate the variation of sampling replicates.

Table 6-4 Rates of DOC biodegradation during the four incubations. The rates were calculated for three phases: fastest, slower and slowest, ascribed according to the BDOC loss (Fig. 6-7). Numbers in the brackets represents which days the rates were calculated for. The resolution for the Hach DO meter was 0.01 mg l^{-1}, equivalent to 0.004 mg l^{-1} C.

	Phase	D-FF1 (mg l^{-1} day^{-1})	D-WF1 (mg l^{-1} day^{-1})	WL13 (mg l^{-1} day^{-1})
Sept. (autumn)	Fastest	0.17 ± 0.01 (Day 0-3)	0.11 ± 0.00 (Day 0-3)	0.12 ± 0.01 (Day 0-3)
	Slower	0.09 ± 0.01 (Day 4-14)	0.05 ± 0.01 (Day 4-14)	0.07 ± 0.00 (Day 4-10)
	Slowest	0.05 ± 0.00 (Day 15-21)	0.03 ± 0.02 (Day 15-21)	0.03 ± 0.00 (Day 11-21)
Nov. (early winter)	Fastest	0.08 ± 0.01 (Day 0-3)	0.05 ± 0.01 (Day 0-7)	0.05 ± 0.01 (Day 0-7)
	Slower	0.05 ± 0.02 (Day 4-21)	0.02 ±0.02 (Day 8-21)	0.04 ± 0.02 (Day 8-21)
	Slowest	/	/	/
Jan. (late winter)	Fastest	0.09 ± 0.01 (Day 0-3)	0.17 ± 0.00 (Day 0-3)	0.13 ± 0.01 (Day 0-3)
	Slower	0.05 ± 0.01 (Day 4-14)	0.14 ± 0.05 (Day 4-14)	0.10 ± 0.02 (Day 4-14)
	Slowest	0.03 ± 0.01 (Day 15-21)	0.08 ± 0.01 (Day 15-21)	0.06 ± 0.03 (Day 15-21)
May (summer)	Fastest	0.19 ± 0.05 (Day 0-3)	0.19 ± 0.02 (Day 0-7)	0.16 ± 0.03 (Day 0-7)
	Slower	0.08 ± 0.02 (Day 4-21)	0.10 ± 0.02 (Day 4-21)	0.08 ± 0.03 (Day 4-21)
	Slowest	/	/	/

6.4.5.2 [SRP] and [TON]

There was little temporal variation in [SRP] of each site during the incubation ($p > 0.05$; Fig. 6-8). However in January a slight decrease was observed in both D-FF1 and WL13 in the first week (from 34.8 ± 2.0 to 27.9 ± 0.5 ug l^{-1}, and from 55.6 ± 1.0 to 47.7 ± 0.6 ug l^{-1} respectively).

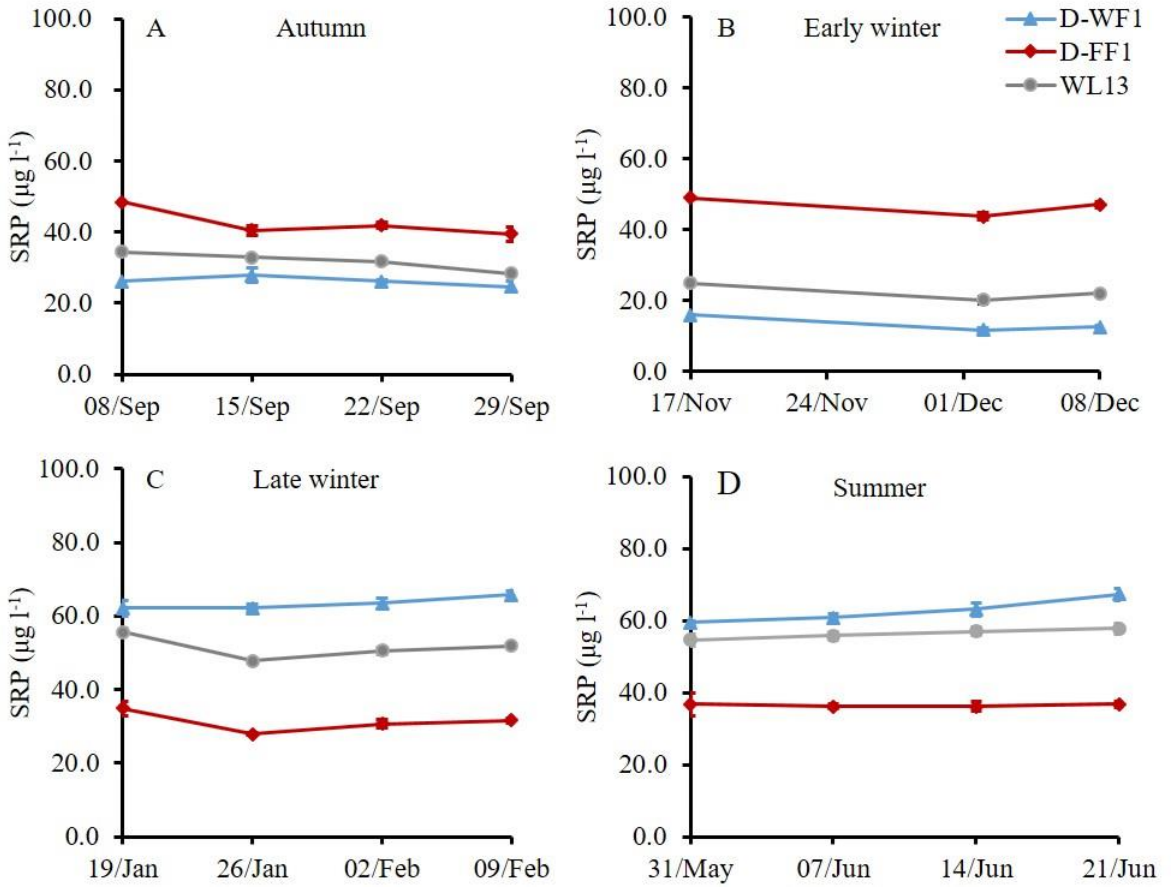


Fig. 6-8. [SRP] changes of samples during each incubation from all sites. The error bars (± 1SD) indicate the variation of sampling replicates. The detection limit for [SRP] analysis was 0.15 µg l^{-1}.

Both spatial and temporal changes were observed in [TON] during each incubation (Fig. 6-9). The concentrations either stayed the same or increased in all bar the November incubation. In September, there was no [TON] detected in D-FF in the first two weeks, but the concentrations increased to 9.9 ± 1.1 ug l^{-1} in the last week. [TON] of D-WF increased from 12.2 ± 6.1 to 29.5 ± 9.9 ug l^{-1} after a slight decrease in the first week. [TON] in the November incubation decreased in all sites, most apparent in D-FF (although there was no data after the first week due to a practice mistake).

In both January and May incubations, [TON] of D-WF increased (Jan.: from 109.3 ± 2.0 to 677.7 ± 23.8 ug l^{-1}, May: from 158.3 ± 1.7 to 581.1 ± 52.3 ug l^{-1}). However the pattern of increase was not the same. In January [TON] remained the same in the first week and then increased rapidly in the second week. In May the [TON] increased most in the first week and slowed down afterwards. D-FF1 showed a different response, with only small increases from 117.9 ± 1.8 to 196.1 ± 7.4 ug l^{-1} in January, and 24.9 ± 9.7 to 115.7 ± 25.7 ug l^{-1} in May. Greater [TON] was measured in D-WF than D-FF for all incubations after three weeks.

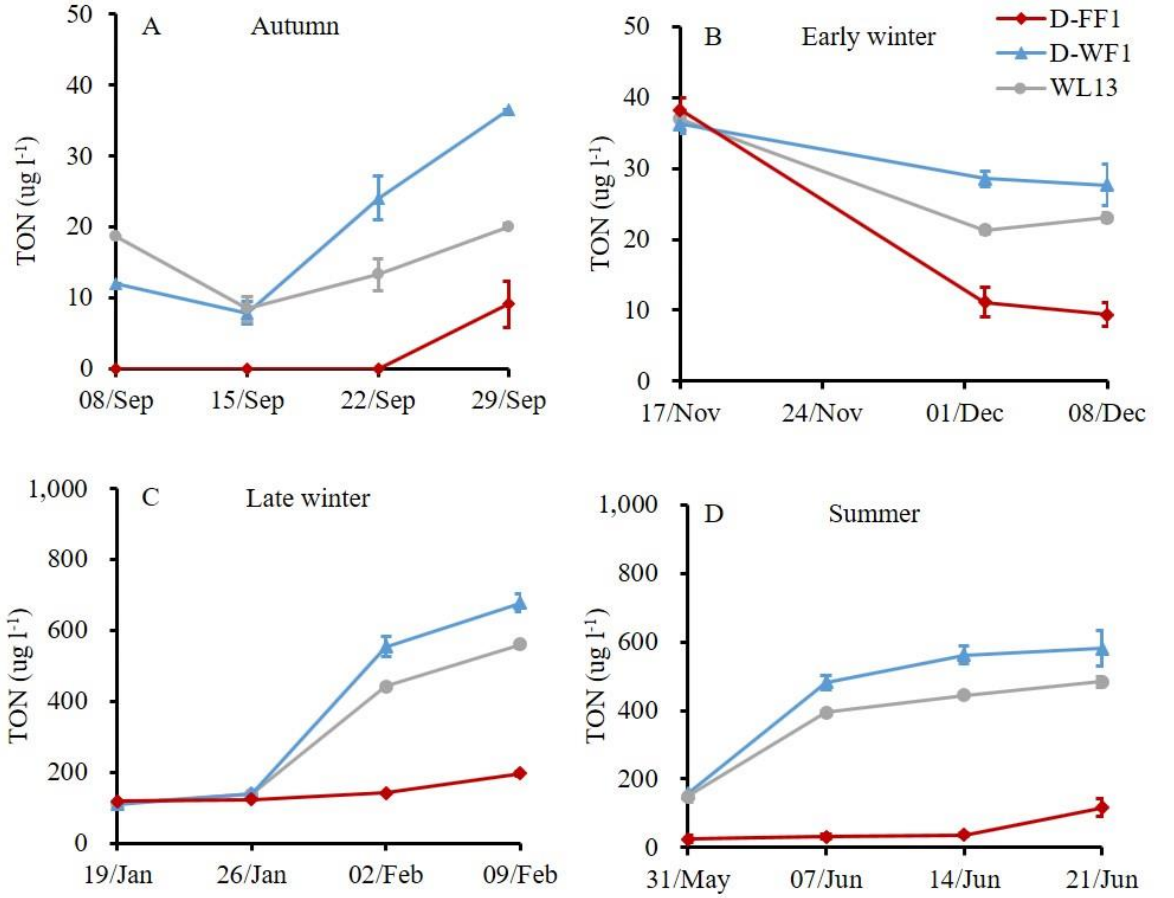


Fig. 6-9. [TON] changes of samples during each incubation from all sites. The error bars (± 1SD) indicate the variation of sampling replicates. Limit of detection for [TON] analysis was 0.33 µg l^{-1}.

6.4.5.3 UV-visible spectrophotometric composition

Although there were intra-site differences and seasonal variation in starting composition, most UV-visible parameters (SUVAs and E ratios) showed little change during the incubations (Fig. 6-10 and Fig. 6-11). $SUVA_{254}$ and $SUVA_{410}$ remained constant over the 21-day incubation in all months bar May. In the May incubation, decreases in D-WF1 and WL13 SUVA values were observed on day 14 before returning to similar previous values. A similar pattern but with a smaller decrease was apparent in the May D-FF1 SUVAs.

E_2/E_4 of all sites was also constant in most incubations (Fig. 6-11 upper). However in the September incubations, an increase was observed for all sites, and more obviously in D-WF1 and WL13 samples (D-WF1: 6.5 ± 0.1 to 6.9 ± 0.0, $p < 0.05$; WL13: 6.7 ± 0.0 to 7.0 ± 0.0, $p < 0.05$). In November, although E_2/E_4 of D-WF1 was stable, a decrease of E_2/E_4 occurred in D-FF1 samples, from 7.8 ± 0.01 to 7.5 ± 0.02 ($p < 0.05$).

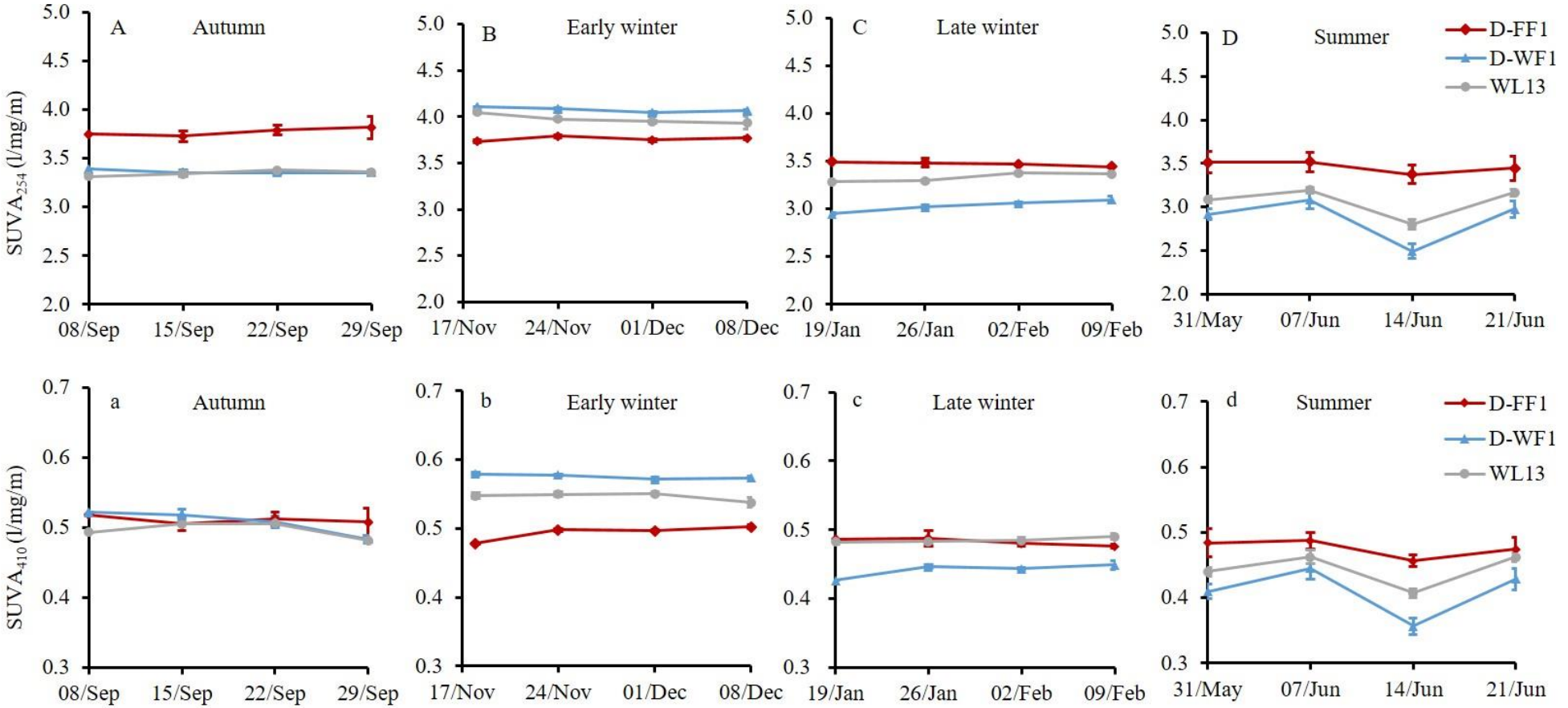


Fig. 6-10. Mean $SUVA_{254}$ (A, B, C and D) and $SUVA_{410}$ (a, b, c and d) changes during the incubations of Drumtee streamwater collected in Sept. and Nov. 2015, and Jan. and May 2016. The error bars (± 1SD) represent the intra-replicate variation.

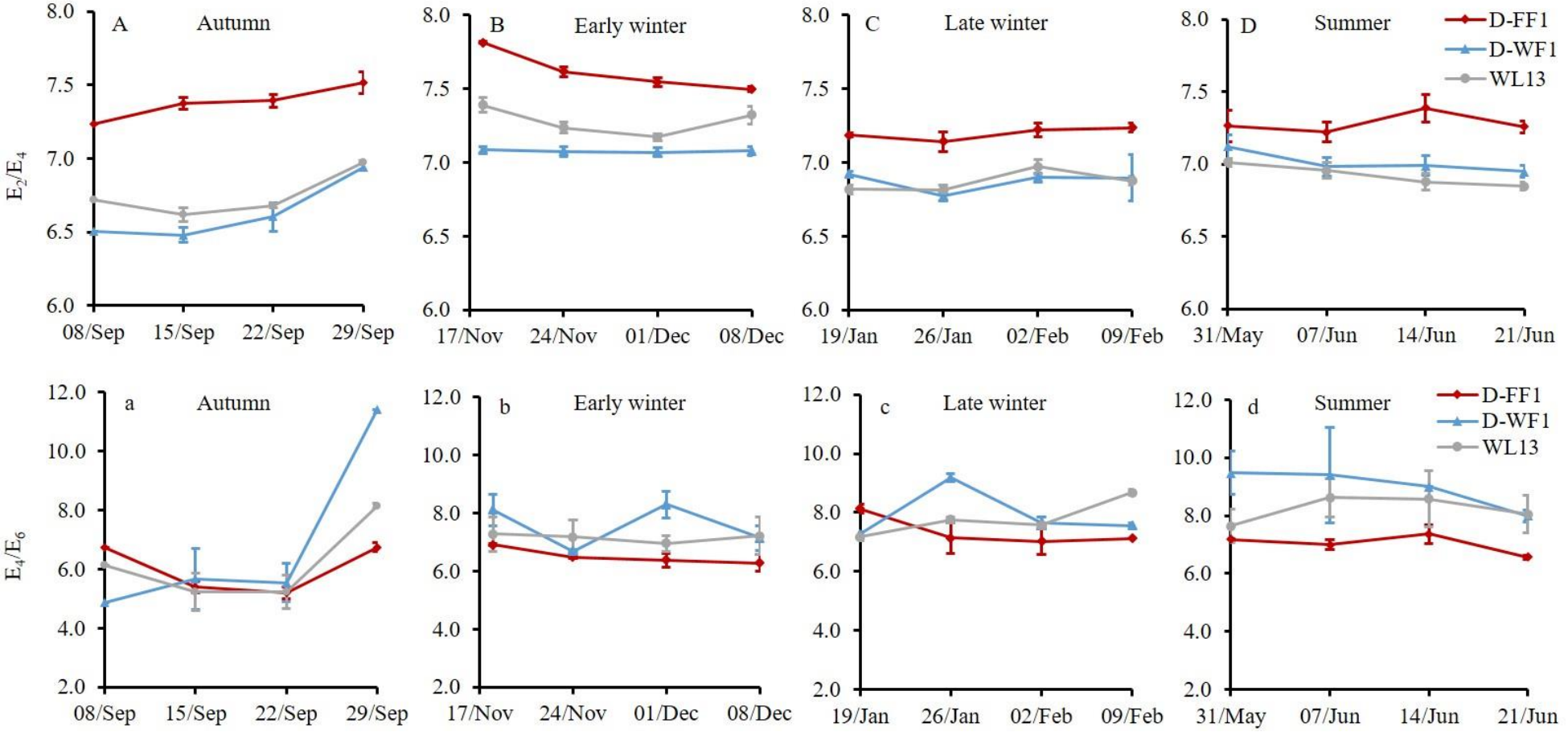


Fig. 6-11. Mean E_2/E_4 (A, B, C and D) and E_4/E_6 (a, b, c and d) changes during the incubations of Drumtee streamwater collected in Sept. and Nov. 2015, and Jan. and May 2016. The error bars (± 1SD) represent the intra-replicate variation.

D-WF1 E_4/E_6 showed variable patterns during the incubations across months but was more constant in the other two sites. Generally D-FF1 E_4/E_6 decreased slightly in Nov. (from 6.9 ± 0.03 to 6.3 ± 0.29, $p < 0.05$), Jan. (from 8.1 ± 0.15 to 7.1 ± 0.00, $p < 0.05$) and May incubations (from 7.2 ± 0.03 to 6.6 ± 0.06, $p < 0.05$). But in September, D-FF1 E_4/E_6 decreased first before increasing from day 14 (increasing from 5.2 ± 0.19 to 6.7 ± 0.16). Meanwhile in the D-WF1 incubation a larger E_4/E_6 increase was observed (from 4.9 ± 0.0 to 11.4 ± 0.0, $p < 0.05$).

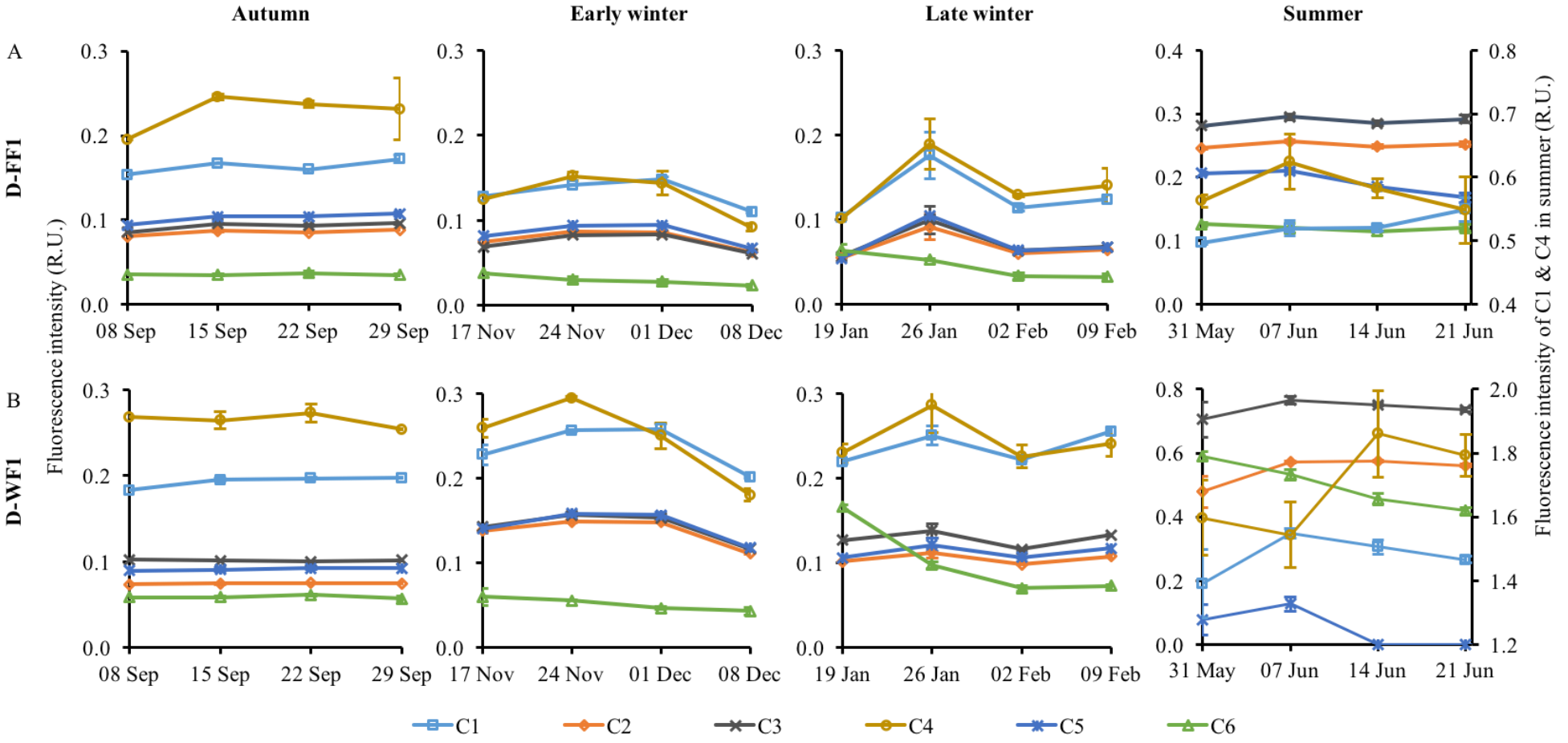
6.4.5.4 Fluorescence spectrophotometric composition

In the autumn (September) incubations, all six components showed little variation during the 21 days, except C4 in D-FF1 increased in the first week from 0.20 ± 0.00 to 0.25 ± 0.00 R.U. (Fig. 6-12A, B and C). C1 and C4 remained dominant at the end, and C6 still had the smallest fluorescence intensities.

The November (winter) incubations of three sites showed more variation in the C1-6 component fluorescence intensities. The humic-like components C1, 2, 3 and 5 exhibited a similar change where the intensity at the end of the incubation was less than the start. However, it was not a linear decrease, rather all sites tended to show a similar increase in mid-incubation. C4 responded differently to the other humic-like fluorophores, decreasing from the second week after the increase in the first week. The C4 decrease was most obvious in WL13 samples, from 0.32 ± 0.00 – 0.17 ± 0.01 R.U. A constant but slight decrease was observed in C6 in all sites.

Component fluorescence was more variable in the January (spring) and May (summer) incubations. In January incubations, C1-5 exhibited a similar changing trend in all sites, increasing in the first week but decreasing afterwards, before a slight increase in the last week, most obvious in C1 and C4. In D-WF1 and WL13, C2, C3 and C5 exhibited less variation during incubation. Only C6 decreased continuously during all incubations. This was most apparent in D-WF1 (from 0.17 ± 0.00 to 0.07 ± 0.00 R.U.), then WL13 (from 0.14 ± 0.00 to 0.07 ± 0.00 R.U.) and the least in D-FF1 (from 0.06 ± 0.01 to 0.03 ± 0.00 R.U.).

Little variation in C1-C5 % was observed during incubation. A clear change occurred in C6 %, but the trend was similar to C6, the absolute value. Therefore the relative contribution of each component is not shown.


Autumn
Early winter
Late winter
Summer
A
B
D-FF1
D-WF1
Fluorescence intensity (R.U.)
Fluorescence intensity of C1 & C4 in summer (R.U.)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1.2
1.4
1.6
1.8
2.0
08 Sep
15 Sep
22 Sep
29 Sep
17 Nov
24 Nov
01 Dec
08 Dec
19 Jan
26 Jan
02 Feb
09 Feb
31 May
07 Jun
14 Jun
21 Jun
C1
C2
C3
C4
C5
C6

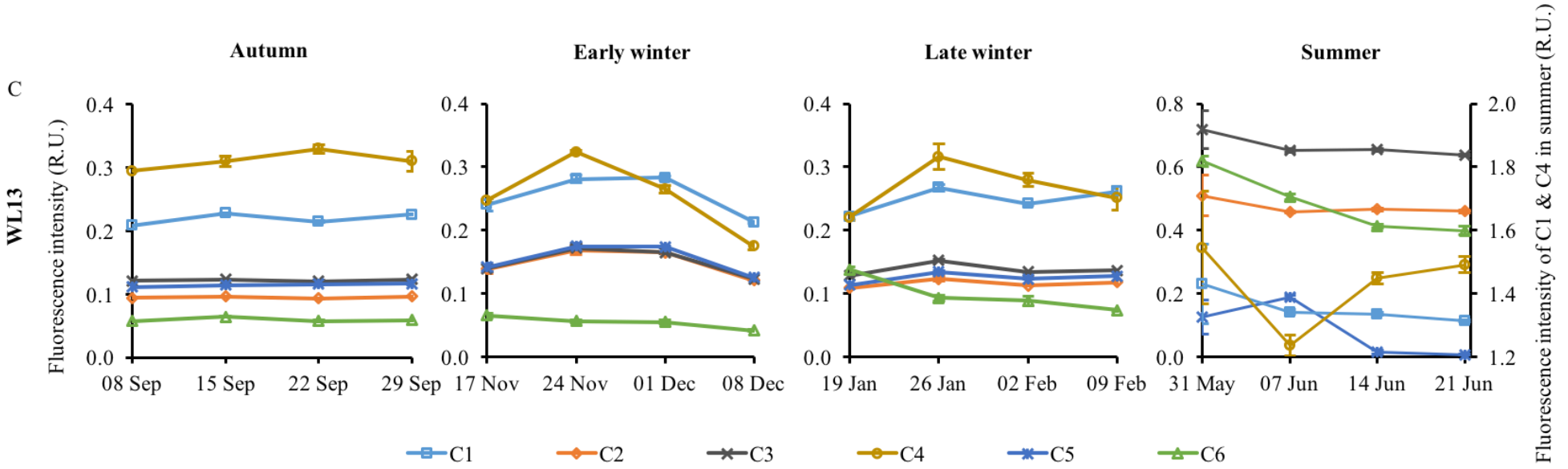


Fig. 6-12. Changes of the six fluorescent components in all sites during each incubation. In the summer incubations, the fluorescence intensity of C1 and C4 were much higher than other components, and are presented on the secondary axis. The error bars (± 1SD) indicate the variation of sampling replicates.

Different patterns and greater variation were observed in the May (summer) incubations. C1, C2 and C3 showed a similar change for each site during the biodegradation process (noting C1 had much higher intensity and was shown on the secondary y-axis), and generally in both D-FF1 and D-WF1 the intensities at the end were slightly larger than the initials. C5 behaved differently to C2 and C3 in May whereas they were similar in other months. C5 of D-FF1 showed a mild decrease along the incubation (from 0.21 ± 0.00 down to 0.17 ± 0.01 R.U.). However in the D-WF1 samples, after a slight increase in the second week, C5 had disappeared by the third week, and in WL13 it approached nearly to zero from the third week onwards. The dominant C4 had varied with no consistent patterns among sites. However, in D-WF1 and WL13, C4 generally increased when C5 decreased. In D-FF1 C6 hardly changed during the incubation (decreased from 0.13 ± 0.00 to 0.11 ± 0.00 R.U., and increased to 0.12 ± 0.01 R.U. at the end). In D-WF1 and WL13 C6 showed large and continuous decreases (from 0.59 ± 0.01 to 0.42 ± 0.01, and 0.62 ± 0.02 to 0.40 ± 0.01 R.U. respectively).

6.4.6 BDOC indicators

When all samples were considered together, some of the characteristics of the fluvial DOM composition showed significant correlations with both the final BDOC/BDOC % (Fig. 6-13 and Fig. 6-14, Table 6-5).

Initial [SRP] was a strong predictor of BDOC loss, with a positive relationship (Fig. 6-13A), explaining 78.4 % of the variation. A similar positive relationship was observed between [SRP] and BDOC %, although less significant ($p < 0.01$, Fig. 6-14A and Table 6-5). [TON] also exhibited a positive correlation, but more significantly with final BDOC % ($p < 0.001$; Fig. 6-14B) and less so with BDOC ($p < 0.05$, Table 6-5).

Both initial $SUVA_{410}$ and $SUVA_{254}$ showed negative significant relationships with absolute BDOC loss ($p < 0001$; Fig. 6-13B and C), but $SUVA_{410}$ had more explanatory power ($SUVA_{410}$: $R^2 = 0.740$; $SUVA_{254}$: $R^2 = 0.677$). The situation differed with BDOC %, and although still negatively related, the relationship for $SUVA_{254}$ was better described by an exponential curve whereas linear for $SUVA_{410}$. Further, $SUVA_{254}$ explained more of variation in BDOC % than $SUVA_{410}$, with greater p and R^2 values (Fig. 6-14C and D, Table 6-5).

Initial C6 was positively and logarithmically related to final BDOC loss (Fig. 6-13D), showing a significant correlation ($p < 0.01$). C6 had relatively less explanatory power for BDOC variation ($R^2 = 0.621$), compared to [SRP], $SUVA_{410}$ and $SUVA_{254}$. The relationship between C6 % with final BDOC% loss became more significant and with greater explanatory power with C6 % ($p < 0.001$, $R^2 = 0.662$, Fig. 6-14E, Table 6-5).

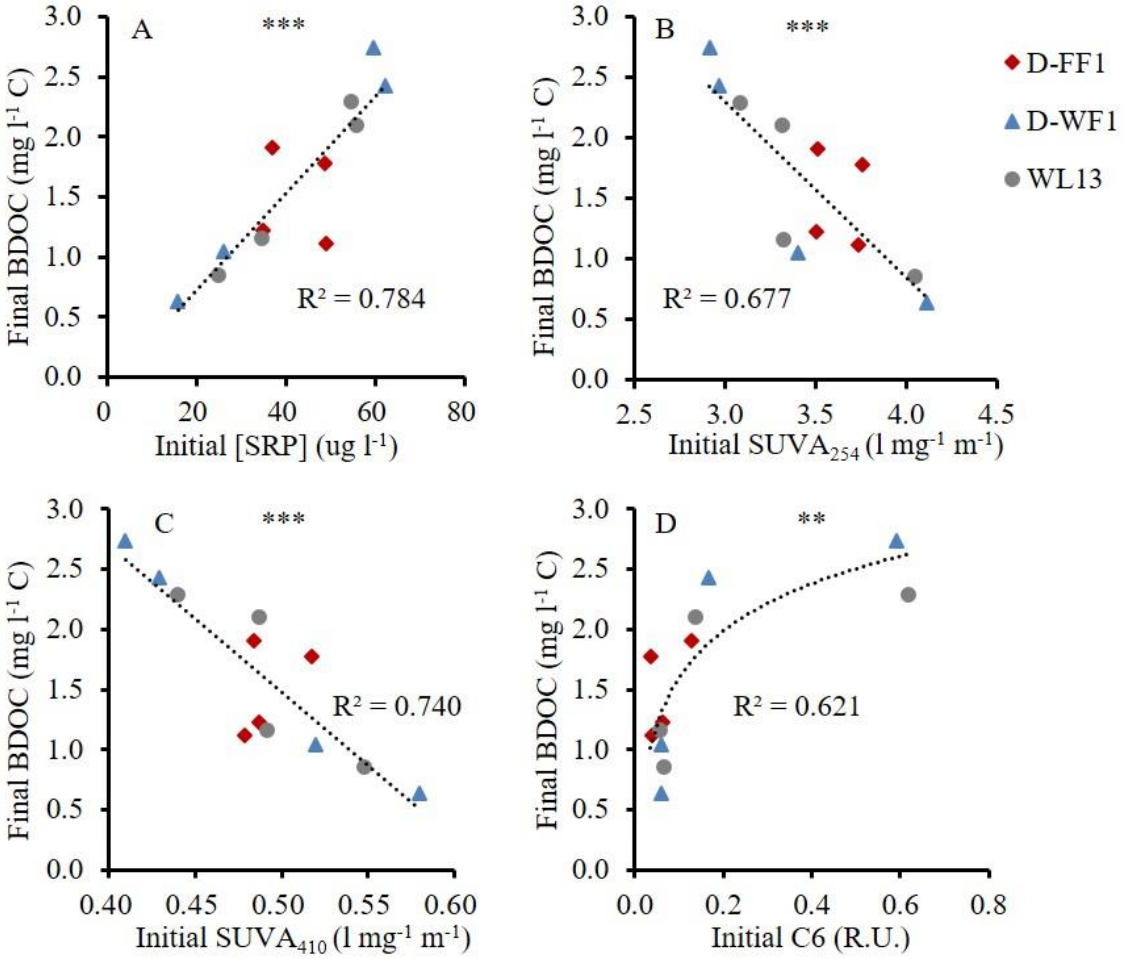


Fig. 6-13. The relationships between initial (A) [SRP], (B) $SUVA_{254}$, (C) $SUVA_{410}$ and (D) C6 fluorescence intensity and final BDOC loss after 21-day incubation, with A, B and C showing relationships more linear, and D being better described by a logarithmic curve. The data has been pooled here to consider if there is a generic response across sites and over time. Each sample point represents the mean composition of the replicates. Only relationships with $p < 0.01$ were presented in the graph. **: $p < 0.01$, ***: $p < 0.001$.

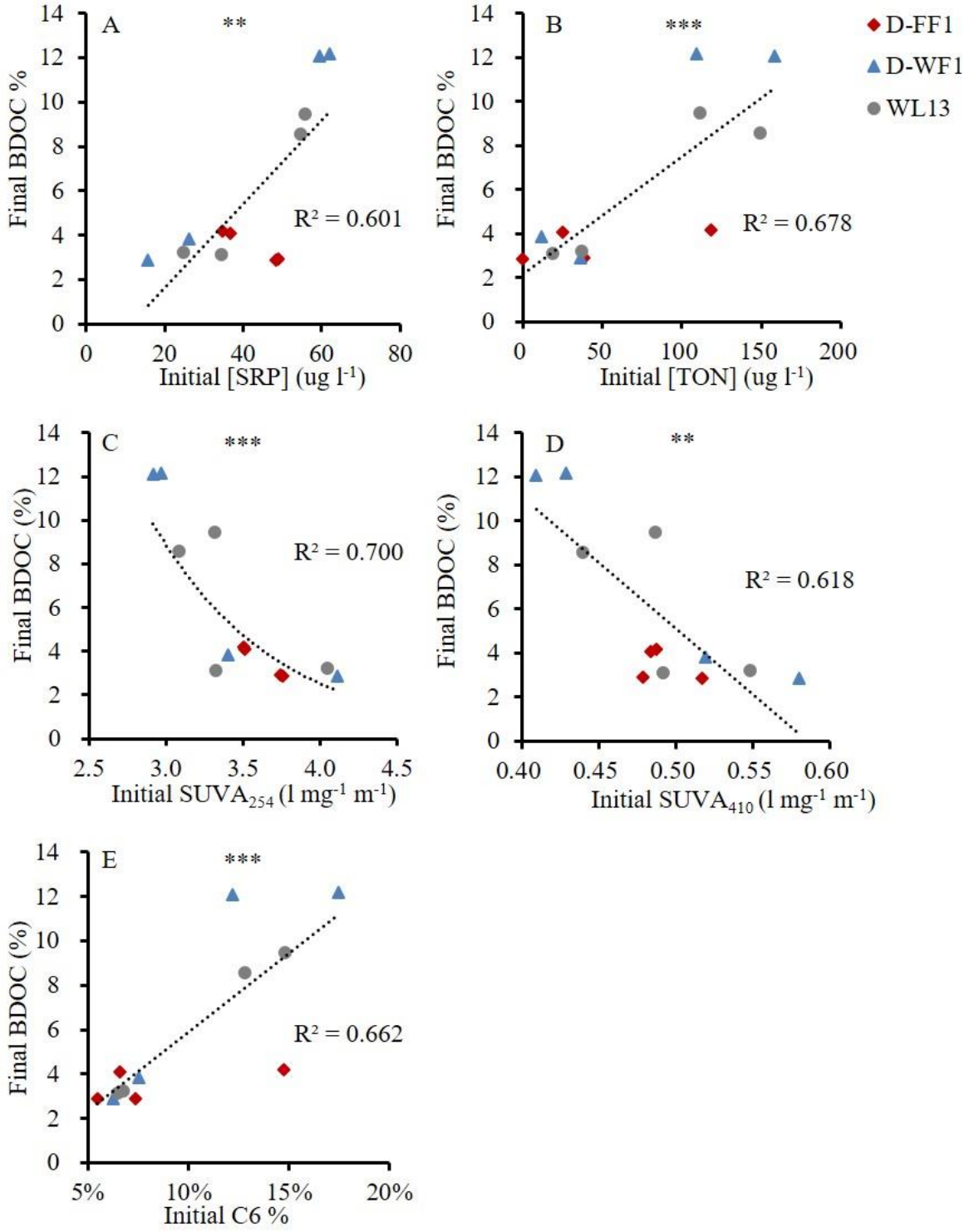


Fig. 6-14. The relationships between initial (A) [SRP], (B) [TON], (C) $SUVA_{254}$, (D) $SUVA_{410}$ and (E) C6 %, and the total BDOC % at the end incubations, with A, B, D and E showing relationships more linear, and C being better described by an exponential curve. The data has been pooled here to consider if there is a generic response across sites and over time. Only relationships with $p < 0.01$ were presented in the graph. **: $p < 0.01$, ***: $p < 0.001$.

Table 6-5 The correlation between the final BDOC/BDOC % and initial [SRP], [TON], and all optical parameters. All initial incubated samples have been pooled here. Absolute fluorescence intensities of C1 – C6 were correlated with BDOC, and percentage C1% - C6% with BDOC %. The R^2 value is not given if the relationship is not considered significant. *: p < 0.05, **: p < 0.01, ***: p < 0.001.

	Final BDOC (mg l^{-1})		Final BDOC %	
	p	R^2	p	R^2
SRP	***	0.784	**	0.601
TON	*	0.457	***	0.678
$SUVA_{254}$	***	0.677	***	0.700
$SUVA_{410}$	***	0.740	**	0.618
E_2/E_4	0.651	/	0.336	/
E_4/E_6	0.26	/	0.192	/
C1 (C1%)	*	0.417	0.183	/
C2 (C2%)	*	0.383	*	0.339
C3 (C3%)	*	0.415	0.575	/
C4 (C4%)	*	0.415	0.842	/
C5 (C5%)	0.981	/	*	0.483
C6 (C6%)	**	0.621	***	0.662

Among the rest of the parameters (including E ratios, C1 – C5 and C1% - C5%), neither E_2/E_4 nor E_4/E_6 were significantly correlated with BDOC or BDOC %. Significant correlations were exhibited between initial C1 – C4 with BDOC, and only initial C2 % and C5 % with BDOC %, but all with smaller significance ($p < 0.05$).

None of the above initial water chemistry parameters could alone fully explain the variance of BDOC/BDOC %. Hence the interaction between water chemistry characteristics and BDOC/BDOC % was further explored using multiple linear regression of the pooled data which are significantly correlated with final BDOC or BDOC as described above. For BDOC, the characteristics [SRP], $SUVA_{254}$, $SUVA_{410}$ and C6 were examined in the multiple linear regression model, and [SRP] and C6 merged as final explanatory variables and were suggested to explain 86 % of the variance of BDOC changes. The residuals were randomly distributed:

$$BDOC = 0.031 * [SRP] + 1.174 * C6 + 0.087 \ (R^2 = 0.86, p = 0.000) \quad \textbf{Equation 6-1}$$

For BDOC %, [SRP], [TON], $SUVA_{254}$, $SUVA_{410}$ and C6 % were examined in the multiple linear regression model, and [SRP] and $SUVA_{254}$ merged as final explanatory variables to together explain 80 % of BDOC % variance. The residuals were randomly distributed:

$$BDOC\ \% = 0.032 * [TON] - 4.57 * SUVA_{254} + 19.46\ (R^2 = 0.80, p = 0.001) \quad \textbf{Equation 6-2}$$

6.5 Discussion

6.5.1 BDOC loss in Drumtee catchment

The incubations of seasonally-collected and compositionally different stream samples showed variability in BDOC loss, from 0.60 – 2.74 mg l^{-1} C (1.57 ± 0.68 mg l^{-1} C). In the September and November incubations, more BDOC loss occurred in D-FF1 than D-WF1 and WL13, but BDOC % was similar across sites (Fig. 6-3). However this inter-site variation changed in the January and May incubations, with more BDOC being degraded in D-WF1 and WL13 than D-FF1. In these two months a larger proportion of D-WF1 and WL13 DOC was biodegradable than was D-FF1 DOC, although [DOC] was smaller in these two sites.

This absolute C loss in Drumtee water was smaller than incubated samples from soil/stream water in bog and forested wetland, and waste water effluent, which ranged from ~3 to 7.6 mg l^{-1} C (Table 6-6). However, this loss was of a similar magnitude to some other sites sampled and incubated under different conditions, such as the incubation with water from Yukon River (0.2 – 2.8 mg l^{-1} C, incubated for 28 days at 15 °C), and stream water in Alaska upland forest (1.4 mg l^{-1} C, incubated for 30 days at 25 °C). It was also close to BDOC of the incubated headwater DOM from a Scottish moorland (1.9 mg l^{-1} C, incubated for 41 days at 15 °C).

The samples from Drumtee had much greater [DOC] (28.1 – 42.2 mg l^{-1} C) than most of other sites (< 20 ml l^{-1} C), and in turn a smaller proportion of DOC is biodegradable, represented by the lower BDOC %. Drumtee BDOC % ranged from 2.9 – 12.1 % (5.8 ± 3.6 %), while most of other site incubations were from ~ 10 % - ~ 40 % (Table 6-6). The BDOC % in this study is similar to those laboratory incubations with headwater draining Austrian catchments with various peat cover (1.63 – 9.30 %), groundwater from Canadian mineral wells and headwater from an American forested coastal plain (Hosen et al., 2014) (Table 6-6).

Table 6-6 The summary of C flux from DOC biodegradation incubations with water collected from various aquatic environments. The summary is presented from the smallest initial [DOC] to the largest. All incubations were conducted in the dark. T represents temperature.

Sampling location	Initial [DOC] (mg l^{-1})	BDOC (mg l^{-1})	BDOC (%)	Duration (day)	T (°C)	Reference
Groundwater in forested areas, Carolina	1.1	0.2	14	42	23 – 26	Shen et al., 2015
Headwater streams draining catchments with different peat cover, Austria	1.41 – 24.31	/	1.63 –9.30	20	18	Fasching et al., 2014
Water from the Yukon River	2.1 – 10.0	0.2-2.8	3-51	28	15	Wickland et al., 2012
Stream water from discontinuous permafrost in Alaska	3	0.5	16	40	25	Balcarczyk et al., 2009
Catchment stream water in upland forest, Alaska	5.8	1.4	23	30	25	Fellman et al., 2009
Stream water from a small lowland stream	6.36	2.2	34	42	20 ± 0.5	Trulleyová and Rulík, 2004
Surface water in forested areas, Carolina	7.3	2.1	29	42	23 – 26	Shen et al., 2015
Reconstituted moorland headwater DOM isolates in Scotland	10	1.9	19	41	15	Stutter et al., 2013
Catchment stream water in bog, Alaska	17.5	3.2	18	30	25	Fellman et al., 2009
Catchment stream water in forested wetland, Alaska	18.7	3.1	13	30	25	Fellman et al., 2009
Waste water effluent	19.5	7.6	39	60	25	Saadi et al., 2006
Drumtee catchment stream water, Scotland	*20.0 – 62.0 (31.4 ± 12.6)*	*0.60 – 2.74 (1.57 ± 0.68)*	*2.9 – 12.1 (5.8 ± 3.6)*	*21*	*20*	*this study*
Bog soil water from coastal catchments, Alaska	27.1	7.3	27	30	25	Fellman et al., 2008
Mineral wells with groundwater input, Canada	28.3	/	4.8 ± 4.2	11	17.5	Olefeldt et al., 2013
Forested wetland soil water from coastal catchments, Alaska	32.1	7.4	23	30	25	Fellman et al., 2008
Lake waters with catchment rich in peat, Canada	35.7	/	0.1 ± 2.7	11	17.5	Olefeldt et al., 2013
Peatland wells with groundwater input, Canada	71.3		-0.5 ± 2.5			
Forested coastal plain headwater streams, America	/	/	~ 5	28	20	Hosen et al., 2014

The difference in BDOC/BDOC % estimated between this study and others may be attributed to the length of DOC incubation (and possibly the way of estimating BDOC), and different DOC biodegradation dynamics (which is discussed in detail in section 6.5.2).

Incubation duration: Drumtee water samples were incubated for 21 days as after this no obvious biodegradation was observed in preliminary trial experiments (Method development, Section 3.2), and there was little dissolved oxygen left in these four incubations (often for D-WF1 incubations, sometimes [DO] was < 1 mg l^{-1}). In other studies, this has ranged from 11 – 60 days (Table 6-6). There is a positive relationship between incubation duration and final BDOC % (produced using data from Table 6-6, BDOC % was chosen as more data was available to boost the relationship) (Fig. 6-15), suggesting that in general BDOC % in dark incubations tends to increase with longer incubation days. The mean BDOC % of Drumtee water is similar to other research of a similar incubation duration and fits with the general pattern. In the Drumtee incubations after 21 days the daily DOC biodegradation rate was not approaching zero on Day 21 (Table 6-4), which suggests that if the samples were incubated for longer and without [DO] limitations, BDOC % would increase.

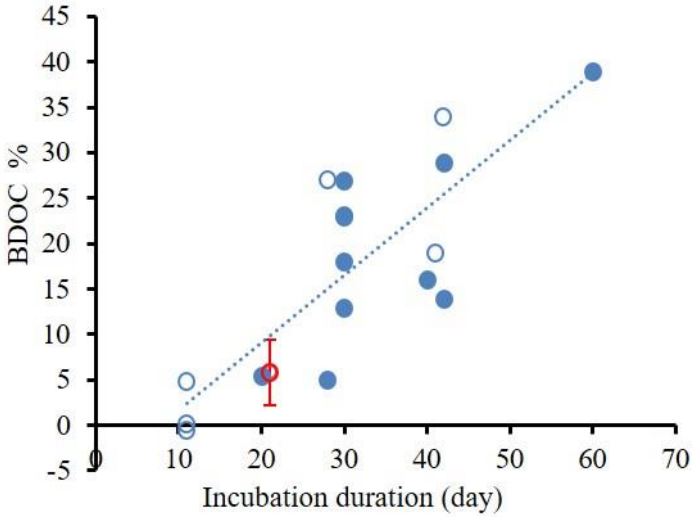


Fig. 6-15. The relationship between incubation length and total BDOC % in dark incubations, according to data from literature review (Table 6-6). Mean values of BDOC % were used. Medium values were implied where the range of BDOC % was given. The red spot represents the mean (± 1SD) from this study. The filled symbols represent incubations where inoculum was applied and BDOC % was estimated from [DOC] change.

BDOC measurement: In the studies listed above, some measured [DOC] changes during incubation for BDOC loss estimation. This may give rise to a concern about overestimation of BDOC loss, as DOC loss caused by absorption to particle surface or incorporation into microbial biomass (if samples are not filtered before incubation, or inoculum was applied), would also be in the estimate of microbial consumption (Trulleyova and Rulik, 2004). In

the Drumtee water incubation, only initial [DOC] was measured. In Fig. 6-15, most incubations having greater BDOC % used [DOC] change for BDOC estimation, when inoculum with soil, sediment or microbial community was added into incubation samples. This may also explain why my experiments have less DOC loss than most of the research listed in Table 6-6.

6.5.2 Biodegradation dynamic during incubation

To understand BDOC loss in Drumtee the influence of DOC quality (form optical properties) and nutrient availability changes can be explored.

6.5.2.1 BDOC and protein-like C6

The three-stage pattern of BDOC loss during the 21 days (fastest, slower, slowest), can be compared to changes in the other parameters that showed most change (Fig. 6-16).

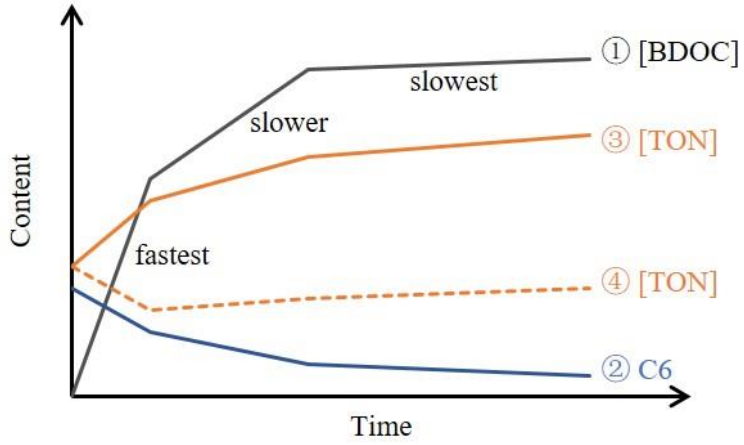


Fig. 6-16. A model exhibiting biodegradation dynamic of DOM during incubation in dark and sealed environment. Both Line ③ and ④ represent the dynamic change of [TON], but two possibilities respectively. [SRP] is not presented as no significant changes were observed during the incubations.

Initial fastest period of BDOC loss: All Drumtee samples had the fastest degradation phase in the first three days, a response also observed elsewhere (e.g. Stutter & Cains 2016). The fast degradation may suggest utilisation of more labile DOC (e.g. amino acids) at beginning than later on, perhaps limited by a reduced labile pool size. The uptake of labile materials in the early part of the incubation is supported by the fluorescence PARAFAC results, where component C6, considered to represent protein-like DOM (Table 6-3), showed a decrease in intensity in the first one or two weeks for all incubations except autumn one (Fig. 6-12). This decrease in C6 intensity slowed down in the later stage of incubation for most sites. The positive and strong relationship between final

BDOC loss and initial C6 was also statistically significant ($p < 0.01$, $R^2 = 62$ %), suggesting C6 availability influences C loss. The relationships between BDOC and humic-like components (C1 – 4) were of less significance ($p < 0.05$) and smaller explanatory power (R^2 = ~ 40 %). Tryptophan-like DOM fluorescence (TFDOM), which likely is a main component of C6, was found to be a good descriptor of rates of heterotrophic bacterial metabolism, and represents a product of bacterial activity as well as a bioavailable substrate (Cammack et al., 2004). This incubation result is consistent with reports that protein-like fluorescent material represents the most bio-labile fraction of fluorescent DOC and likely has significant contribution to a large proportion of BDOC (Lapierre and Del Giorgio, 2014; Stedmon and Markager, 2005).

However, in the September incubations, C6 showed little variation while 0.6 – 1.8 mg l^{-1} final BDOC was processed and the fastest phase was observed for all sites (Fig. 6-7). As a single predictor, C6 (or C6 %) only explained 62 % (or 66 %) of total absolute (or relative) BDOC loss. However, other labile DOC components with simple composition, e.g. sugars and peptides, do not absorb light or fluorescent and so will not be identified by PARAFAC (Stubbins et al., 2014). Indeed, a proportion of DOC has been found to be non-chromophoric, non-humic and thus optically invisible to UV-visible spectroscopy, and can range up to 89 % of total DOC (Pereira et al., 2014). Further, the labile low-molecular-weight (LMW) compounds such as carbohydrates and carboxylic acids are increasingly recognised as important drivers of bacterial productivity in surface waters. For example, acetate was identified as responsible for 45 % of bacterial respiration of LMW carbon in northern Sweden (Berggren et al., 2010). Therefore it is likely that proteins (as identified by C6) and other non-fluorescent lower molecular weight molecules account for significant parts of the faster BDOC loss.

Furthermore, the decreasing C6 intensity in the fastest phase of incubations (mainly in November and January ones) was accompanied by a concurrent increase in intensity of most humic-like components (Fig. 6-12). The protein-like peak has been observed to consist of a mixture of proteinaceous materials and phenolic moieties in humic-like substances (Maie et al., 2007). Therefore, the corresponding inverse relationship between humic-like components and C6 may suggest that a) the breakdown of C6 released the fractions such as phenolic moieties, which directly contributed to the humic-like increase, or b) some humic-like components may be derived from microbial decomposition and transformation of dissolved protein materials during the incubation, to form the humic substances at the early stage of DOC humification (e.g. C2 was reported to be microbial-transformed (Cory and Mcknight, 2005). This latter interpretation supports the perception of the dual role of microorganisms as consumers and producers of DOM (Guillemette and

Giorgio, 2012), and that bacteria utilise primarily aliphatic molecules (Trulleyova and Rulik, 2004).

Middle slower and later slowest DOC loss: The initial faster stage may deplete the biodegradable DOC and other semi-labile components become the major BDOC source. This can be seen in the continuous albeit slow decrease in C6 from Day 7, and a decrease in some humic-like terrestrial components (e.g. C4 and C5 in most incubations) from later of incubations (Fig. 6-12). In the November incubations, when all BDOC loss occurred at the slower rate from days 14 – 21 (Fig. 6-7), all humic-like components (apart from C4) showed decreased in intensity, while little change was detected in C6. This supports an interpretation that semi-labile DOC was used during this period. Although the final slowest stage was not reached in my incubations when stopped by Day 21, in the hypothesized model, BDOC loss rate decreases at this phase, influenced by reduced availability of DO and/or lack of biodegradable DOC.

6.5.2.2 Nutrients

SRP: For every 1 mg l^{-1} C consumed, microbes need 40 ug l^{-1} N and 8 ug l^{-1} P to satisfy growth requirements, when assuming a bacterial growth efficiency of 0.4 and a bacterial molar ratio for C:N of 10 and C:P of 50 (Fellman et al. 2008). The range of BDOC lost across the incubations (0.60 – 2.74 mg l^{-1} C) would require about 5 to 22 ug l^{-1} [P] in total. The initial [SRP] was greater than the maximum [P] required (except D-WF1 in winter, Table 6-2), and thus was sufficient to support this C consumption during the 21 days without other P inputs. However, no obvious change was observed in [SRP] during the 21-day incubation (therefore is not described in the model) (Fig. 6-8). This may either indicate that SRP production offset uptake, or that bacteria were using a different source of P, possibly dissolved organic P which was made available as DOC was processed.

TON: As a major form of dissolved organic N, the protein-like components (tyrosine-like and tryptophan-like) are thought to be both produced and consumed by microorganisms (Maie et al., 2007; Williams et al., 2010). Thus it may be expected that as DOC is consumed there may be changes in [TON], and considering these can help understand further key processes in the incubations. Two patterns in [TON] changes with time were observed (Fig. 6-9) and described by lines ③ and ④ (Fig. 6-16), which are thought to arise depending on whether N is available or limited.

N is not limited: At beginning of incubations where N is not limited, the release of more N from DOC (mainly C6) decomposition than is used by bacteria contributes to an increase

in [TON]. The [TON] increase slows as less C6 becomes available and/or there is more microbial respiration. Finally, this concentration increase almost stops as N uptake equals release, due to limited DO for respiration and little microbial production and consumption (Fig. 6-16, line ③). This pattern is typical of D-WF1 and WL13 incubations in summer (Fig. 6-9D), where the large [TON] increase in the first week (D-WF1: from 158.3 up to 481.6 ug l^{-1}, WL13: 149.1 up to 393.6 ug l^{-1}) might be a result of more N release than consumption.

N is limited: Here the microbial uptake of N is larger than the release from DOC, and leads to a decreasing [TON] in the first stage of the incubation (described as line ④), for example in the September (apart from D-FF1) and November incubations (Fig. 6-9). Here initial [TON] was small and ranged only from 11.9 – 38.2 ug l^{-1}. [TON] of D-FF1 in September was below limit of detection and this likely also reflects more N consumption than production. The decrease in [TON] slows down to stabilise at a lower but sustained concentration as microbial processes release fresh N (e.g. the November incubation), or an increase in [TON] can happen if more N is released than being consumed (e.g. the September incubation).

6.5.3 Controls on BDOC loss in Drumtee catchment: nutrients and DOC composition

The loss in BDOC appears to be potentially influenced by initial nutrient dynamics and DOC chemical composition. Both BDOC and BDOC % were correlated with SRP, SUVAs and C6 respectively, with the greater significance ($p < 0.01$) than other parameters, whereas BDOC % was also significantly related to TON with $p < 0.001$ (Table 6-5). When taken all parameters into account, [SRP] and C6 together explained 86 % of BDOC variance (Equation 6-1), while [TON] and $SUVA_{254}$ explained 80 % of BDOC % changes (Equation 6-2).

6.5.3.1 Nutrients

SRP: Although [SRP] stayed constant during incubations, a significant and positive relationship between initial [SRP] and total BDOC (or BDOC %) loss was observed (Fig. 6-13, Fig. 6-14), suggesting that phosphorus availability may be linked to DOC biodegradation. This makes sense as P is recognised to accelerate bacterial growth rate and efficiencies (Smith and Prairie, 2004), and so a bigger community may be able to process more DOM. Therefore when more initial [SRP] was available, this likely supported more bacterial production, and so more DOC could be degraded. For example, in D-WF and WL13 more BDOC (and BDOC %) was processed in January and May than September and November

(Fig. 6-3) and this is also when starting [SRP] were 2-3 times higher (Table 6-2, Fig. 6-2B). There was little variation in [SRP] in D-FF1 across the year and similarly there was little variation in BDOC processing.

TON: A significant relationship between N and BDOC has been observed elsewhere (e.g. Wickland et al., 2012). In Drumtee catchment, a positive relationship was observed for initial [TON], but more significantly with BDOC % ($p < 0.001$) although less so with absolute BDOC ($p < 0.05$, Table 6-5). The dynamic changes of TON during incubations further support this close link between TON and DOC biodegradation. TON may have less influence on absolute BDOC loss than other parameters mentioned above (including [SRP], SUVA and C6). This was witnessed in the incubations. For example, in November, although the initial [TON] across all sites was greater than that in September, less DOC was degraded, and this was concurrent with the decrease in initial [SRP] in D-WF and WL13, although not D-FF (Table 6-2). The less impact from [TON] than [SRP] on BDOC was further supported in the multiple linear regression analysis, where [TON] was not chosen. With these observations, it is possible to suggest in Drumtee catchment, where nutrients concentrations are low, phosphorus seems to be a more important macronutrient linked to absolute BDOC loss than nitrogen.

6.5.3.2 DOC composition

Among all spectroscopic parameters considered for DOC composition (SUVAs, E ratios and PARAFAC components), SUVAs and C6 were more significantly correlated with BDOC (and BDOC %). It is not surprising that C6 influenced BDOC degradation, either individually or with [SRP], with the close dynamic links during incubation as discussed earlier (section 6.5.2). Thus the following section presents mainly the possible controlling impacts from SUVAs.

Both initial $SUVA_{254}$ and $SUVA_{410}$ showed significant but negative relationships with BDOC (and BDOC %) consumption for all sites (Fig. 6-13 and Fig. 6-14), suggesting DOC is more biodegradable when it is less aromatic. In January when there was similar [DOC] in all sites, the greater starting aromaticity with lower [SRP] in D-FF than the other two sites, may account for the lower consumption of DOC (Table 6-2). The impact of DOC aromaticity has been observed elsewhere. For example, in the 90-day incubation of soil-derived DOM extraction from Norway, samples with low $SUVA_{280}$ (around 0.7 – 1.6 l mg m^{-1}) showed high mineralization with the maximum around 90 %, but those with high $SUVA_{280}$ (around 3.1 – 4.4 l mg m^{-1}) showed only approximately 5 % degradation (Kalbitz et al. 2003). In-stream DOC mineralization decreased with increasing residence time and

$SUVA_{400}$ (a proxy for more-complex aromatic C, Chapter 4) in a UK peat catchment (Worrall et al., 2006) indicating DOC aromaticity limited C biodegradation.

However in most Drumtee incubations, all SUVAs were observed as stable during the incubation indicating the fraction of humic substances did not change much (Fig. 6-10). Only in the summer incubation was a decrease observed in $SUVA_{254}$ and $SUVA_{410}$ across sites, and more so in D-WF and WL13. SUVA has been observed to change little in other incubations (e.g. Kalbitz et al., 2003; Olefeldt et al., 2013). Microbial degradation is considered to preferentially target molecules with low molecular weight and low aromaticity (Wickland et al., 2007), while coloured, photoreactive molecules with high molecular weight and aromaticity are mainly decomposed by photochemical degradation (Stubbins et al., 2010). It could be possible that SUVA of Drumtee water changed little as the less-labile aromatic molecules were not the major fraction of BDOC degraded during the 21-day dark incubations, where photodegradation was not happening. Instead, clear changes were observed in C6, which may suggest fluorescence PARAFAC is a sensitive method to detect the small changes of BDOC for the humic water samples.

The potential control of DOC composition on BDOC loss helps to understand the spatial variation of DOC biodegradation between the two Drumtee tributaries. In January and May, the greater DOC biodegradability in D-WF1 may be influenced by the larger proportion of C6 and smaller aromaticity than that in D-FF1. The often smaller starting SUVA of D-WF DOC (thus likely more biodegradable) than D-FF was the opposite to DOC composition in Drumtee observed during 2014 – 2015, where $SUVA_{410}$ was constantly larger in D-WF (Chapter 4, Fig. 4-11). This seems contradictory, but there are two possible reasons for this. Firstly, in the time series of the annual observations (see discussion in Chapter 4, Section 4.5.4), the greater value in $SUVA_{410}$ for D-FF, more similar to this incubation study, are generally coincident with drier periods. Stream water for the incubations was collected in January (frozen ground) and May (approaching summer) when flow was reduced and so groundwater was also likely proportionally more important, and DOC in D-WF was more labile than D-FF due to the different carbon sources. Secondly, as Drumtee Water is still receiving run-off impacted by wind farm-related land use changes, DOC compositional characteristics may differ with time, for example as less forest brash exists to be degraded. DOC SUVAs during the incubations from 2015 – 2016 effectively extend the time series of DOC spectrophotometric composition, and suggest longer monitoring may be needed to allow the consideration of land use impacts on DOC composition.

6.5.3.3 Additional: water residence time

Although the impacts of water residence time on BDOC loss was not examined in this study, it is necessary in the future research to take this into account, as the longer residence time may increase the possibility of DOM photodegradation. It has been suggested that photodegradation can modify DOM function, selectively degrading aromatic and phenolic groups (Spencer et al., 2009), and photo-oxidation and photo-cleavage can decrease OM lability considerably (Barry et al., 2016; Bertilsson, 2000). For example, in incubations using water from a UK peatland, but in ambient light conditions, [DOC] declined by 64 % (from 42 to 17 mg l^{-1} C) over 70 hours compared with 6 % decline (from 42 to 36 mg l^{-1} C) for the sample incubated in the dark (Moody 2016). Although the Drumtee BDOC % fits the pattern showed in Fig. 6-15, and is similar to other peaty water incubation results, to some extent the small Drumtee BDOC in this study may be due to the absence of DOM photodegradation and there is potential that this study underestimates the biodegraded C loss in real aquatic systems. This could be an idea for next stage of this DOC biodegradation experiment.

6.5.4 Seasonality of Drumtee BDOC loss

With water samples collected in autumn, early winter and late winter in 2015, and summer in 2016, the four incubation results in this study provide a preliminary understanding of the potential for BDOC consumption in Drumtee water, that is dependent on the source DOM and macronutrient composition: there was more BDOC (and BDOC %) loss from Drumtee in late winter and summer but less in autumn and early winter (Fig. 6-3). However it should be noted that this observation was from incubations carried out at 20 °C, while the fluvial temperature varies with season and can be considerably lower in the winter.

However, the greater BDOC (and BDOC %) loss in January and May incubations, has been observed elsewhere across different aquatic environments. For example, a larger BDOC % in incubated UK moorland stream water was observed in February (Stutter et al. 2013). In the bog and forested wetland in Alaska, the greatest BDOC % loss happened with stream water DOC in the May runoff (Fellman et al., 2009). Further, the greatest BDOC % uptake from a Yukon River water incubation was also measured in January, followed by early spring freshet in May (the spring thaw and flood event) (Wickland et al. 2012). All these incubations were carried out at different temperatures. This suggests that the seasonality of BDOC loss in Drumtee water is similar to other aquatic systems, and that apart, from temperature, there are other controlling factors that have more influence on this seasonality.

From the discussion in section 6.5.3, it is suggested nutrient concentration is one of the main factors affecting DOC biodegradability in Drumtee water. According to the long-term survey, [TON] showed a general seasonal pattern with the greatest concentration around from December to February (Chapter 4, Fig. 4-16). This may support the more BDOC degradation in the January incubation. Moreover, at the time of sampling in January the surface ground was frozen and covered by snow. So the samples were more groundwater-dominated and thus maybe more nutrient-rich and less aromatic (supported by the decreased $SUVA_{254}$ in D-WF1 and WL13). The low temperature in the field would limit microbial processing and nutrient uptake in stream. Thus when this more labile and nutrient-rich DOC samples was moved to lab and incubated at 20 °C, the bacteria might become more active and had resources to respond. This was reflected by enrichment in initial TON and the protein-like component C6 % (Fig. 6-2 and Fig. 6-6). Therefore in January, the greater amount of BDOC loss can be partly attributed to the greater abundance of more easily accessible C fractions (substrates) and SRP and TON (nutrient). Furthermore, it has been suggested that DOC recently flushed from catchment during storm events has more labile and semi-labile BDOC pools than that from baseflow (Stutter and Cains, 2016), as more fresh terrestrial DOC inputs. In Drumtee, more storm events were observed in winter/spring. Although DOC aromaticity and thus resistance (represented by SUVAs) did not show clear seasonal variations during annual survey, it is worth trying to analyse temporal variation of the labile DOC pool (represented by C6) in Drumtee in the future research.

6.6 Conclusion

Inland aquatic systems receive great amount of terrestrial-derived DOM, which is traditionally thought to be largely resistant to microbial metabolism (Fasching et al., 2014; Ward et al., 2013). However this traditional perception is changing, and this study together with other research support those ideas that terrestrial DOM might represent an important component of bioavailable C pool that respired in rivers (Fellman et al., 2008; Mann et al., 2012). In January and May, with decreasing [DOC] and increasing nutrients at most sites in Drumtee, there was an increase in BDOC loss. Although DOC in Drumtee was observed to be more humic and aromatic than many other aquatic systems, the higher [DOC] in Drumtee streams (Chapter 4) contributed to the in-stream C loss similar to many other systems when normalised for the length of the incubation.

In Drumtee, nutrient availability, especially phosphorus, and DOM composition (as characterised by protein-like C6 and SUVAs, appeared to be the characteristics most importantly related to the rate of DOC biodegradation. Greater P concentrations initially

allowed bacterial growth, and thus larger composer community, but the lack of change in [SRP] may suggest organic P also have been important. Nitrogen was less significantly related to BDOC but more so to BDOC % loss, mainly as contributing to the nutrient supplement. However, there does seem to be a metabolic link between TON and DOC (especially protein-like fluorescent component and a certain humic-like components) during DOM biodegradation. When more TON was available, more protein-like materials were likely formed, which is suggested to be microbial-labile. The decomposition of this protein-like component may have released or be transformed to humic-like components, produced during the early stage of DOM humification. Thus increases were observed in some humic-like components during incubations. This process further shaped DOM to become more refractory. Protein-like C6 was significantly and negatively correlated with BDOC loss, and showed a clear decrease during most incubation, suggesting protein-like materials are important BDOC pools. $SUVA_{254}$ and $SUVA_{410}$ appeared to have had significant and negative impacts on biodegradation, with more biodecomposition when DOM was less aromatic and humified.

Land use impacts on DOC biodegradation via changing DOC composition were less clear in this sub-project. From the earlier study in Chapter 4, it is known that land use differences from windfarm construction and deforestation in the Drumtee catchment likely affected DOC composition in the draining water, with the larger HS content and more resistant DOC thought prevalent for D-WF (indicated by larger $SUVA_{410}$ and smaller E_2/E_4, Chapter 4). However, the inter-stream difference of DOC $SUVA_{410}$ in the incubations was different to that observed before in the spatial composition study (however the patterns for [DOC] and other UV-parameters were broadly the same). This may be a result of 1) the incubated DOC samples were from one site of each tributary and showed variation of composition to the averaged DOC composition in Chapter 4; 2) the DOC sources became different in the two streams when the water table may be low in January and May indicating reduced surface run-off.

The multiple regression models based on the incubation results can help to reconstruct the time series of BDOC loss in Drumtee catchment. However it is not possible to do this at this stage, as 1) C6 was not routinely measured at any site in Drumtee, and 2) nutrients had only been monitored at confluence site and so these are not considered further in the discussion. However, these equations indicate the more likely controlling factors on BDOC degradation in this catchment, which is helpful for conducting future research to further explore how land use difference exerts impacts on the in-stream BDOC degradation.

7 Conclusion

7.1 Introduction

More commonly research in the terrestrial-aquatic carbon cycle has focused on quantifying DOC export from peatland to surface waters, considering controlling factors such as land use disturbance (e.g. Hergoualc'H and Verchot, 2011), and the composition of exported DOC has been less explored. Yet, DOC composition influences its reactivity and interactions with other stream components (e.g. nutrients and key micronutrient), and the decomposition processes in surface waters. The overall objective of this research was to understand the importance of aquatic DOC composition to the fluvial carbon cycle, and how land use influenced this. This research consists of three sub-projects investigating:

1) wind farm-related disturbance to peatland water and impacts on DOC concentration and composition (Chapter 4);

2) total dissolved [Fe] in peatland water and the interactions with DOC and nutrients (Chapter 5);

3) biodegradability of compositionally-different peatland DOC (Chapter 6).

This concluding chapter first addresses how the specific objectives of this research (more detail in Chapter 1) were fulfilled (7.2). Subsequently how these research findings have elicited future research needs is identified (7.3), and the key contributions this research has made to the wider-knowledge base is considered (7.4).

7.2 Summary of key findings

Objective 1*. Explore how long disturbance by wind farm development affects water chemistry in peaty catchments, by analysing the long-term trends in fluvial DOC and nutrient concentrations in streams draining the wind farm.*

This was addressed mainly by considering the ten-year long-term monitoring time series (July 2006 to September 2016) of DOC concentration and export in the five peatland catchments draining the south part of the UK's largest onshore wind farm, Whitelee wind farm. [SRP] and [TON] were also considered as changes in these nutrients can indicate disturbance to macronutrient cycles.

Significant increases were observed in [DOC] in all catchments across the ten years ($p < 0.05$ in Kendall Seasonal analysis, Table 4-2), which is different to previous conclusions that [DOC] could return to the original value five years after development (Phin, 2015)

One possibility may be the observed decrease by Phin (2015) could be the annual variation of [DOC] with time. [DOC] increases show greatest variation within year at WL13 and 15 during construction periods and with patterns independent from other Whitelee catchments (Fig. 4-2). WL13 and Wl15 were most affected by the original and extension developments respectively. This suggests that wind farm construction disturbance may be an important driver of [DOC] increase pulse in the Whitelee catchments during the construction periods. Without a control site in this long-term monitoring to measure the background changes (e.g. climate impacts, acid deposition), it is difficult to interpret if land use change is the main reason for the increased [DOC]. However, as wind farm wind development (both deforestation and construction) was the most intensive land use management in Whitelee during the monitored period, it is still reasonable to suggest land use change, apart from the potential background change impacts, may have contributed to the increased [DOC]. This suggestion is further supported by observed spatial and temporal variation in [DOC] within the Drumtee catchment (see key findings in the discussion here of Objective 2), and the rapid [SRP] increase at WL13 and 15 during the original and extension development periods respectively (Fig. 4-14).

However, no year-on-year increasing trend was observed in the DOC export during the hydrological years 2011 – 2016. Instead, there was a broadly similar trend of DOC export to annual discharge, indicating controls on DOC export to streams and fate within the water body are more complex, and closely linked to climatic conditions. The wind farm development may still exert long-term impacts on C loss, by land responses which generate more DOC in terrestrial stores for export - but how much is transferred to the fluvial system depends on run-off patterns.

No consistent long-term impact on [SRP] was found. Concentrations did increase immediately after disturbance, but recovered approximately one year after the development finished, indicating limited temporal influence of wind farm construction. Small but significant decreases in [TON] across years were observed in all catchments bar WL15, which showed an increase in 2011 (one of the extension years) (Fig. 4-16 and Table 4-7). The net nitrate consumption within the catchment after deforestation may be one of the reasons contributing to the general but small [TON] decrease in Whitelee.

Taken all together, the above results suggest wind farm-associated disturbance may be long-lasting in the perspective of increasing in-stream [DOC]. Therefore, with the sensitivity of peatland to climate changes and anthropogenic disturbance, and the large amount of carbon stored in peat, careful consideration should be made for wind farm development in peatlands.

***Objective 2**. Assess if different wind farm-associated land uses, that is wind farm construction and deforestation, are another control on the spatial variation of fluvial DOC quantity, and importantly, DOC composition.*

DOC quantity: The impacts of different wind farm-related land uses on DOC concentration and composition were analysed by considering two sub-catchments, D-WF and D-FF, in the Drumtee catchment (WL13) during 2014 - 2015. The Drumtee catchment had [DOC] (5.2 to 89.3 mg C l^{-1}) comparable to peatland catchments in UK (e.g. Jones et al., 2016; Moody and Worrall, 2016; Pawson et al., 2012; Worrall et al., 2007), but at the higher end of the range. Spatial variation was found within Drumtee. D-WF, the stream draining the wind farm area, had consistently smaller [DOC] (6.6 ± 0.7 – 49.0 ± 4.0 mg l^{-1}) than D-FF, the felled sub-catchment (13.5 ± 1.4 – 81.6 ± 9.5 mg C l^{-1}) (Fig. 4-5). With the similar peat coverage, topography and vegetation across the Drumtee catchment (Chapter 2, Section 2.1.2), it is likely the [DOC] variation was linked to different land uses. Disturbance caused by road construction, peat excavation and turbine installation may expose deeper peat soil and release more 'aged' DOC. However the spatial variation suggests deforestation contributed to a greater [DOC] increase in the adjacent streams, which may be related to the decomposition of tree litter and dying tree roots after felling activities (Zerva and Mencuccini, 2005). In addition, the inorganic nutrients released from tree litter (Kreutzweiser et al., 2008) and possible higher soil temperature without tree canopy (Jauhiainen et al., 2012) may facilitate organic matter decomposition. Further, migration of DOC from peat soil to draining water in this Fe-rich catchment (Table 5-2) could be affected by oxidation of Fe with changed soil aeration. If there was more aeration of the deeper peat (rich in soluble Fe^{2+}), through installing the turbines, there could be DOC co-precipitation with Fe oxyhydroxides at redox interface (see discussion in Chapter 5) and thus smaller D-WF [DOC] in streams.

DOC quality: While no clear seasonal pattern of DOC composition variation was observed at large Whitelee catchments and small Drumtee sub-catchments, DOC composition varied between sites. Between Whitelee catchments, DOC from the Drumtee confluence water (WL13) was less humic (indicated by smaller $SUVA_{410}$ and larger E_2/E_4, $p < 0.05$) than the other catchments (Table 4-4). As WL13 is the smallest catchment sampled, this difference may reflect less in-stream DOC degradation (both photoreaction and biodegradation) due to shorter water residence times, and less nutrients (particularly nitrate) in Drumtee. However, water samples were collected from the outlets of each catchment and here more than windfarm construction may influence the DOC pool. Thus it was difficult to unpick the potential influence of wind farm-related land uses on inter-catchment DOC composition variation. Therefore, this was further explored within WL13

Drumtee, the smallest catchment with sampling close to the areas of disturbance by considering two sub-catchments, one dominated by felling, D-FF, and one without felling and more turbines, D-WF.

Fluvial DOC composition differences were observed between the Drumtee sub-catchments. DOC aromaticity and HA:FA were similar between two sub-catchments, indicated by the similar $SUVA_{254}$ and E_4/E_6 (Fig. 4-9, Fig. 4-12 and Table 4-5). However, D-FF DOC seems to have less HS (smaller $SUVA_{410}$; larger E_2/E_4) than D-WF (Fig. 4-10, Fig. 4-11 and Table 4-5). It appears that DOC exported from D-FF was 'fresher' and less humified than D-WF. Leachates from freshly-decomposed tree litter are commonly of low molecular weight (Marschner and Kalbitz, 2003), and thus likely 'younger' and less humified DOC in D-FF, whereas DOC released from deeper peat would generally considered to be more resistant due to the accumulation of more-complex HS after labile carbon has been decomposed by microbial (Glatzel et al., 2006; Hogg et al., 1992). Further, drainage may create a more aerobic environment in D-WF deeper peat, and trigger the 'enzymic-latch' which can accelerate further DOC humification (Freeman et al., 2001; Worrall and Burt, 2005). It is less clear which mechanism led to the DOC composition difference between two sub-catchments, or all may happen simultaneously, but in D-WF wind farm construction activities may cause the exported DOC to be more resistant.

DOC composition showed different responses to dry conditions between the Drumtee sub-catchments, with D-WF DOC being less aromatic (indicated by smaller $SUVA_{254}$, Fig. 4-9) and humic (larger E_4/E_6, Fig. 4-12) than D-FF in the dry months. These different responses may be mediated by the different land uses. A relatively higher water table in D-FF than D-WF may be likely due to less drainage and the cessation of evapotranspiration. Aromaticity of pore water DOC first increased then decreased with peat soil depth (Gandois et al., 2013), thus it may be possible that in the drier months, more degraded DOC was exported from deeper peat in D-FF, whereas if water table in D-WF dropped below the zone of maximum accumulation of aromatic carbon, less aromatic DOC may have been exported. The different responses to the drier condition has implications for DOC biodegradation. For example, in water samples collected in ground-frozen and summer periods SUVAs were smaller and more DOC from D-WF was biodegraded than D-FF (Chapter 6). The greater importance of groundwater with less aromatic DOC in D-WF under low flow condition may explain this response.

Overall the above observations suggest land use differences, namely wind farm construction and deforestation, exert influence not only on the DOC concentration in the

draining waters, but also on DOC composition. Felled areas likely produced more DOC, but of smaller aromaticity than DOC in land affected by turbine construction.

Objective 3. *Investigate relationships between total dissolved Fe concentration and concentrations of DOC and nutrients (phosphorus and nitrate), as this interaction may ultimately shape the DOC biodegradability during fluvial transport.*

The total dissolved [Fe], and its interactions with [DOC], [TON] and [SRP] were assessed both between the five Whitelee catchments, as well as between the sub-catchments (D-WF and D-FF) in Drumtee.

The total dissolved [Fe] was high in these organic soil rich catchments (0.15 – 4.27 mg l^{-1} across all sites), which was comparable with the observations in other peaty waters but towards the higher end of the range (Table 5-2). Statistically significant and positive relationships were found between [DOC] and total dissolved [Fe] for all sites (Fig. 5-3, Fig. 5-5 and Table 5-1) regardless of climatic conditions (the driest and the rest periods), indicating a close interaction between DOC and Fe in the peaty waters. This coupled variation was suggested due to the formation of stable Fe-DOC complexes that stopped Fe^{3+} from precipitation and thus increased Fe export (Sarkkola et al., 2013). The higher [DOC] in the Whitelee peaty catchments than many other peatland waters (Chapter 4), makes this an interesting site to explore the interactions between Fe and DOC.

Land use (deforestation), together with peat cover and water retention time, may contribute to the spatial variation in [Fe]. Among these factors, deforestation can lead to [Fe] increase by producing and releasing more [DOC] into the waters (e.g. WL15). However, [DOC] at all sites only explained 42 – 77 % of variance in the total dissolved [Fe], and the correlation strength varied with hydrological changes (the driest sampling periods were different to the other periods) and sites (Table 5-1). Further analysis shows Fe binding ability with DOC (indicated by Fe/DOC molar ratio) significantly increased with decreasing E_2/E_4, and with increasing E_4/E_6 (although this relationship was weaker and only clear in the Whitelee catchments and not observed in D-WF or D-FF) (Fig. 5-7 and Fig. 5-9). This suggests in addition to the spatial variation of [DOC] in this study (Chapter 4), the different DOC composition (Chapter 4), which may be partially attributed by water retention time, also influenced Fe export in the fluvial systems. Indeed, this Fe-DOC interaction goes two-ways.

First, humic DOC may enhance Fe in-stream transport. It was found at all sites more Fe was mobilized per unit DOC when there was a larger proportion of HS in the DOC (Fig. 5-7

and Fig. 5-9). This is because Fe binds with organic ligands, particularly with aromatic structures (Kaiser and Guggenberger, 2000; Kritzberg et al., 2014; Riedel et al., 2013). However Fe may preferentially bind with FAs than HAs as indicated by the positive although weaker relationship between Fe/DOC and E_4/E_6 (Fig. 5-9). This may be because the HAs have larger molecular weight and more complex aromatic structure, which could be less reactive and easy to aggregate with Fe (Kritzberg et al., 2014). Given the observed spatial variation of DOC composition between the Whitelee catchments (Fig. 4-6 and Fig. 4-7, Chapter 4), this may explain the largest Fe/DOC being in WL16 and the smallest in WL13 (Fig. 5-8). Thus it can be suggested that DOC with larger proportion of HS may enhance Fe export from peatlands to fluvial systems, which puts an additional pressure of drinking water treatment works where Fe must also be removed to produce water with and this can a potential threat to drinking water quality as [Fe] in drinking water must be < 0.2 mg l^{-1}.

Second, the high dissolved [Fe] in freshwaters may however limit DOC export. In the oxidising environment, DOM can be removed by coagulation with Fe hydroxides (Riedel et al., 2013). Here, in the driest months Fe/DOC was larger than the suggested precipitation ratio (0.014) in D-WF (Fig. 5-6), and in some Whitelee catchments (e.g. WL16 in July and September 2014, Fig. 5-8). The greater Fe^{2+} supply in deeper soil water/groundwater (Abesser et al., 2006) likely contributed to the larger Fe/DOC. Although DOC may increase the binding ability with a larger proportion of HS in the driest months, with the Fe/DOC close to or larger than 0.14, there may be fewer binding sites and thus a greater chance for Fe and DOC to co-precipitate. This is significant as: 1) the co-precipitation of Fe with DOC may help to explain the smaller [DOC] in the driest months (Chapter 4), and improve current knowledge about DOC loss during fluvial systems; 2) Fe may preserve the terrestrial character of peat-derived organic carbon which would have further biogeochemical implications in oceanic ecosystems; 3) the precipitation may limit DOC biodegradability in waters.

In addition, while only a weak relationship was found between Fe and TON, the significant correlation between Fe and SRP in the Whitelee catchments suggests that that Fe-DOC complex may increase SRP export, as Fe-DOC complexes suppress the formation of solid $FePO_4$ (Sundman et al., 2016). Thus, in peat-rich catchments, the biogeochemical cycles of C, P and Fe are closely-related, as the high concentration and humic composition of DOC exported may not only increase [Fe], but also indirectly increase P availability. However, too much Fe, particularly in the dry periods, may reduce both C and P exports.

Objective 4. *Quantify how much DOC is biodegraded in peatland streams and what controls this.*

This objective was met by carrying out dark biodegradation incubations of Drumtee water samples collected from D-WF, D-FF and the confluence site in four seasons, to consider possible seasonality and spatial variation, and influencing factors.

While Drumtee [DOC] was higher than many other fluvial systems during the annual survey (Chapter 4), and in these four incubations (28.1 – 42.2 mg l^{-1} C), BDOC in Drumtee water only ranged from 0.60 – 2.74 mg l^{-1} C (1.57 ± 0.68 mg l^{-1} C), with the consequently small BDOC % of 2.9 – 12.1 % (5.8 ± 3.6 %). While the absolute BDOC loss is similar to incubated samples from some other systems, e.g. water from Yukon River and from upland forest, the proportion of biodegraded DOC was smaller than many other aquatic systems, which commonly ranged from ~ 10 % - ~ 40 % (Table 6-6). BDOC % was similar to samples from peat-covered headwater, and underground water from forested coastal plain (Table 6-6). However, this may not be a site-specific response. Different incubation conditions (mainly temperature and incubation length) were applied between different incubation experiments and normalised for the incubation length, Drumtee BDOC % loss was comparable with others (Fig. 6-15). This observation supports the idea that aromatic and humic terrestrial DOC can be reactive to biodegradation (Berggren et al., 2010; Fasching et al., 2014; McCallister and del Giorgio, 2012).

Seasonal variation was observed in Drumtee BDOC, with greater DOC biodegradability (indicated by BDOC %) in late winter (January 2016) and early summer (May 2016) and less in autumn (September 2015) and early winter (November 2015) (Fig. 6-3). More DOC was lost via biodegradation in the latter two months, but this was not apparent in D-FF. Spatial variation occurred between sites, but changed in different seasons. In autumn and early winter, more BDOC was lost in D-FF1 than D-WF1 and the confluence site, although BDOC % was similar between sites. Conversely, in late winter and early summer, more BDOC (and BDOC %) loss happened in D-WF1 and the confluence site.

The UV-visible and fluorescence analysis, and nutrient measurements, helped to understand the DOC dynamic during biodegradation. A three-stage pattern of BDOC (fastest, slower and slowest) describes the dynamic changes (Fig. 6-16), with most of the DOC biodegradation in this study similar to the stages one/two. The protein-like component C6 decreased during DOC biodegradation, while other PARAFAC components and UV parameters remained relatively constant (Fig. 6-10, Fig. 6-11 and Fig. 6-12), indicating that C6 may be a significant factor to DOC biodegradability. However semi-

labile components may be used when the most biodegradable DOC (e.g. C6) was less available, supported by the decrease in some humic-like terrestrial components (e.g. C4 and C5) during the later stages of incubation (Fig. 6-12).

While SRP showed no clear change during incubation, TON responded in two different ways to DOC biodegradation, depending on if N was or not limiting in the incubation environment. If N was not limited, the release of more N from DOC (e.g. C6 decomposition) than the consumption by bacteria led to a TON increase. This increase would slow down before reaching a balance due to less availability of C6 and/or more microbial activity leading to less organic N release. Incubations of D-WF1 and WL13 in summer (Fig. 6-9D) typically show this pattern. However, if N was limited at the beginning of incubation, more N may be consumed by bacteria and less released from DOC decomposition. As a result, a decreasing [TON] was observed (e.g. the November incubations, Fig. 6-9B). The decrease would slow down and stabilise at a lower [TON], possibly due to the more N release as a result of more microbial activities (e.g. the November incubations, Fig. 6-9B). These observations during incubations suggest the protein-like component may be an important source for DOC biodegradation, with N materials closely interacting with BDOC during this dynamic process.

The starting composition of incubated DOC and nutrient concentrations were analysed to understand BDOC loss. Nutrient availability (particularly SRP) and DOC composition (represented by protein-like C6 and SUVAs) are the most important to Drumtee BDOC loss. A significant and positive correlation between initial [SRP] and total BDOC (and BDOC %) loss was found (Table 6-5), with SRP alone explaining 78 % (60 %) of BDOC (and BDOC %) variance. This may be linked to the more DOM decomposition by the bigger bacteria community, which can be supported by the more availability of [SRP]. [TON] was more significantly and positively correlated with BDOC % with a stronger relationship ($p < 0.001$, $R^2 = 68$ %), but less so with BDOC ($p < 0.05$, $R^2 = 46$ %). C6 had a positive correlation with BDOC and BDOC %. This is not surprising as the close interaction between BDOC and C6 was observed during incubation. Both SUVAs showed significant and negative impacts, explaining about 60 – 75 % of variation in BDOC (and BDOC %) loss (Table 6-5). This supports the idea that aromatic and humic DOC is less available to bacteria.

These parameters can thus be used as indicators for Drumtee BDOC loss, and help to better explain the BDOC seasonality and spatial variation. For example, in the late winter (January) incubation, less microbial uptake of both nutrients and protein-like C6 at low temperature likely contributed to the greater DOC biodegradability than in autumn and early winter. Further, DOC aromaticity and the proportion of HS in D-WF and WL13

decreased in the January and May compared to November (Fig. 6-4), and this may also lead to the larger BDOC loss. In these two months, groundwater may become dominant supplying less aromatic DOC into draining water (see discussion in Chapter 4). Further, between the two sub-catchments, the greater nutrient concentrations, smaller DOC aromaticity and more labile C6 in D-WF supported the greater DOC biodegradability than D-FF in January and May.

Wind farm-related disturbance (wind farm construction and deforestation) in Drumtee was suggested to influence in-stream DOC reprocessing via impacts on DOC composition (Chapter 4), which however was less evidenced here. However, it is noticed that in three out of four incubations, D-WF DOC had smaller $SUVA_{410}$ than D-FF, which was not typical of the annual DOC composition observed during 2014 – 2015 in Drumtee (Chapter 4), but still within the range. Two possible reasons may be responsible to this. First, as discussed earlier (in Objective 2 section), different responses of DOC composition changes to the decreased water table (namely decreased aromaticity in D-WF and increased in D-FF) were observed in the annual survey, which may be caused by the different carbon sources. This may also happen in January (frozen ground) and May (summer month) incubations, when the flow was reduced and groundwater likely became more dominant. Second, this may reflect the variation of DOC composition from one site of each tributary (D-WF1 and D-FF1) to the averaged value for each sub-catchment. More sampling sites and more frequent incubations are recommended for future research to comprehensively consider land use impacts on BDOC related to spatial and temporal DOC composition variation.

From the incubations, nutrients, C6 and $SUVA_{254}$ were significant parameters of multiple regression models developed for reconstruction time series of BDOC loss in Drumtee. Future research with more frequent analysis of these parameters will be helpful to further examine the influence of land use on in-stream DOC reprocessing.

7.3 Ideas for future research

7.3.1 Direction 1: Land use impacts on DOC export and composition variation

Annual discharge showed an overriding impact on DOC export from the Whitelee catchments, which makes it difficult to unpick the land use disturbance on DOC loss. The hydrological and so climatic controls on aquatic DOC export in peat-dominated catchments are observed elsewhere (e.g. Leach et al., 2016; Olefeldt et al., 2013). To further explore if wind farm development controls DOC export from the catchments, predictive models of DOC dynamics in soil and stream can be applied to simulate DOC

export. Recent model development, such as the process-based catchment model Integrated Catchments model for Carbon (INCA-C) with routine climatic parameter monitoring (de Wit et al., 2016; Futter et al., 2007), and a coupled hydrological-biogeochemical model capable of predicting DOC fluxes through considering both near-surface runoff and baseflow contribution (Dick et al., 2015), allows the simulation of DOC concentration and export with consideration of climatic conditions including precipitation, temperature and hydrological connectivity in the major landscape units. With the simulated DOC exports from the Whitelee catchments, meteorological and landscape drivers (including annual precipitation, temperature, soil pore water [DOC] and different land uses) of annual DOC loss can be evaluated with statistical analysis, and this approach may be helpful to examine the long-term impacts of wind farm development on catchment carbon budget.

Further, limited research has explored the impacts of wind farm development on peatland on DOC quantity, and even less on DOC composition (Chapter 4, Table 4-1). Here given the observed spatial variation of DOC composition in Drumtee likely caused by the land use differences (wind farm construction and deforestation), it is worth further analysis to target understanding of the mechanism behind such land use impacts.

The close connection between terrestrial and fluvial systems is suggested to be dominated by organic-rich riparian zone, which provides the major DOC source entering streams (Ledesma et al., 2015; Strohmeier et al., 2013). However, groundwater source becomes important in the low flow condition. Therefore, DOC composition characterization along the vertical profile of Drumtee riparian zone and mineral soil could be conducted and compared with stream DOC properties. This allows 1) testing of the earlier assumption that changes of DOC aromaticity with soil depth which have been observed in other research also exists in Drumtee peatland; 2) the generation of important information to consider if and what different terrestrial DOC sources (top/deep peat soil water or groundwater) contribute to the streams draining different land uses.

In addition to the UV-visible spectroscopic measurement, fluorescence PARAFAC analysis could also be applied to characterize terrestrial and fluvial DOC. Humic-like components may be used as a tracer for terrestrial DOC, with the knowledge that most humic-like components in this study are land-derived (Table 6-3). Furthermore, radiocarbon and stable carbon isotopes have been used as a powerful tracer to explore and differentiate carbon sources from adjacent environments (Chasar and Chanton, 2000; Lozanovska et al., 2016). A profile of $\delta^{13}C$ values of peat organic matter along the soil depth shows the vertical changes in stable carbon isotope signature (Krohn et al., 2017). $\Delta^{14}C$ has also been

used to differentiate the ages and sources of aquatic organic carbon mobilized from land in e.g. forested and agricultural catchments (Longworth et al., 2007; Lu et al., 2014). These tracing methods can be both used to further explore the carbon source responsible for the terrestrial characters of aquatic DOC in receiving waters with different land uses and under varied flow conditions.

Environmental parameters, specifically pH in peat soil, could also be surveyed, as this has been indicated to significantly affect DOM composition and result in higher-molecular-weight DOC(Roth et al., 2015; Seifert et al., 2016). With a better knowledge of the exported DOC source into streams, and environment changes indicated, for example by pH, the full extent to which land use influences the aquatic C cycle can be understood.

7.3.2 Direction 2: Refine of understanding of DOC biodegradability

Seasonality was observed in the Drumtee BDOC loss. However, only four months were sampled for incubations, leaving the changes of BDOC during the interval periods unknown. Moreover, as discussed in Chapter 6, the incubation temperature was higher than that in the field, especially in winter months. The constant and higher temperature in the laboratory condition could yield differential responses to field BDOC breakdown and its seasonality. Since the approach for BDOC loss analysis developed in this study is simple and sensitive for measuring small breakdown, it may be worthwhile to further:

1) carry out continuous monthly incubations for a year, but with less frequent incubation measurements (e.g. only at the start and end of each incubation) to support this logistically, and generate a more comprehensive knowledge of temporal and spatial variation of BDOC loss. The incubation protocol would be the same to that used in this study. With the more frequent sampling, the developed multiple regression models can be tested and refined, which allows a better prediction of BDOC time series.

2) considerate the temperature influence on DOC biodegradation by conducting incubations at a series of temperatures (e.g. 5, 10, 15 and 20 °C). The other incubations conditions would be the same between different temperature groups.

In addition, Fe impacts on BDOC loss could be further considered. In Drumtee, Fe-DOC complexes likely increase P bioavailability by suppressing the formation of $FePO_4(s)$. However if Fe/DOC is large enough, Fe can also precipitate DOC via coagulation and reduce P availability (see Chapter 5). Both processes could influence DOC biodegradation in the fluvial systems. Indeed, brown precipitation was observed during the incubation.

Thus, apart from the influencing parameters discussed earlier in Chapter 5, the dissolved [Fe] should be measured during the incubation. Further, to refine the understanding of these impacts from Fe, it would be valuable to examine the exact Fe-DOC and Fe-P co-precipitation points for Drumtee water, by adding a series of [Fe] into water samples with the same [DOC] and [SRP]. The understanding obtained can help to explain [Fe] impacts on DOC biodegradation during the annual BDOC survey.

7.3.3 Direction 3: DOC degradation along a river continuum

A decrease in organic carbon reactivity along the continuum of inland waters has been observed, with the suggestion that water retention time controls organic carbon decomposition (Catalán et al., 2016). According to this, a more resistant carbon pool, possibly linked to aromatic and humic materials, would be suggested downstream of the draining streams and rivers. However, the controlling process shaping DOC composition during transport remains less understood.

During the DOC transport along the continuum of freshwater networks, several factors can together shape the compositional characters of DOC. In addition to the biodegradation process, photoreaction is another key process removing in-stream DOC (Stubbins et al., 2010), selectively decomposes coloured, aromatic DOC (Spencer et al., 2009), resulting in a residual pool with smaller proportion of CDOM. However the production of photodegradation would likely fuel autotrophic microbial activities, producing autochthonous and small molecular weight DOC (Jones et al., 2016). With downstream catchments receiving more terrestrial DOC inputs, a mixture of different DOC sources (autochthonous and allochthonous), as well as dynamic decomposition processes of both resistant and labile DOC, making it less clear how DOC composition character changes during transport and what the relevant controls are. Some research has considered DOC reactivity along aquatic systems, but limited attention has been paid to the peatland catchments affected by wind farm development.

Thus assessment of dynamic DOC composition, combined with the measurement of DOC degradability and relative significance of different aquatic DOC sources along a river continuum, would help to understand the science behind the changing DOC reactivity with long water retention time and potential land use impacts. First, DOC degradability can be assessed by considering both biodegradation and photoreaction at multiple sites along the Drumtee mainstream. As the examination of DOC biodegradability is time-consuming, BDOC indicators such as protein-like component C6 (results from Chapter 6) or an improved multiple predictive model from Direction 3 could be used to estimate aquatic

DOC biodegradation by multiple sampling along the river. The same water samples would be analysed for DOC photoreactivity by conducting irradiation incubations in the ambient environment.

Second, DOC spectrophotometric composition of both DOC in the riparian zone, groundwater and river could be analysed and represented by UV-visible parameters and PARAFAC components used in this research. The comparison of DOC composition between different sources would help to indicate the dominant terrestrial DOC source in water, and how the relative importance of terrestrial/autochthonous DOC sources are changing during the water transport, with the integrated influence on aquatic DOC property. Potential controls of land use differences (e.g. turbines and deforestation near headwater, pasture and urbanization downstream) can be analysed for the changing DOC composition.

Third, relationships between DOC degradability and composition parameters could be explored to further consider the impacts of DOC inherent characteristics (photo-reactive and microbial labile) on its in-stream reprocessing over gradients of increasing terrestrial influence.

The in-stream DOC process is complex with changing environment influencing DOC dynamics with other stream components. With the above proposed analysis of DOC reactivity during water transport and potentially changing DOC sources, a better understanding of DOC in-stream dynamics can be generated.

7.4 Research contributions to the wider-knowledge base

This research has focused on the impacts of land use disturbance to peatland, particularly wind farm construction and associated deforestation, on aquatic DOC composition and relevant implications to the fluvial system. The environmental impact assessments on wind farm development on peatlands are still rare, thus this research helps to fill the knowledge gap, especially in the perspective of carbon budget, which can be potentially helpful in future studies. To finish, the main contributions are summarized below:

1. Assessment of wind farm long-term impacts on water quality is lacking, but commercial wind farm construction is still occurring, to meets targets of the lower C emissions in the UK. This research presents ten-years of monitoring of changes in micronutrients concentration in peat-draining fluvial systems, and this is of significant length, so makes a valuable contribution to the existing knowledge base of wind farm development on peatland. The observation of no clear increasing trend in carbon

export from these catchments boosts the current data set of DOC concentration and export changes in the northern peatland regions, which can help to consider the role of peatland in the global carbon cycling.

2. This is only the second study (the other being (Niekerk, 2012)) to show the temporal and spatial variation of aquatic DOC composition with the consideration of impacts from wind farm-associated land uses, suggesting the wind farm disturbance may have changed the quality of the exported DOC. This approach expands biogeochemical understanding of the fluvial ecosystem, particularly the interaction between carbon and trace elements and the DOC reactivity and reprocessing dynamic, both of which were evidenced in this study. The understanding of inter-catchment differences of DOC quality, and the close Fe-DOC interaction also informs our need to assess treatability of both DOC and Fe for drinking water purposes, particularly in peat-rich areas such as the Northern Scotland.

3. This is the first study to explore the impacts of wind farm-associated land uses on DOC in-stream metabolism, by providing a unique comparison of DOC biodegradation from streams draining the turbine-installed and felled sub-catchments. This research and the data collected have provided useful information about in-stream carbon loss which could better inform the comprehensive assessment of net carbon exchange and environment impact of wind farm construction.

4. Estimates of small BDOC loss in the peaty water was calculated by combining incubation using small tightly-sealed reagent bottles and measurement of [DO] consumption. This convenient and parsimonious technique allows the assessment of the biodegradation of humic and recalcitrant peat-derived DOC, and can be applied to other aquatic environments where similar resistant DOC would be expected. In addition, the observed DOC breakdown which was comparable to that in other aquatic systems when normalized by the incubation length, which has not been considered before, rather across site variation was interpreted. This increases understanding of the biodegradability of land-derived DOC, which is still a little fragmented.

5. The weekly observation of DOC breakdown in this research provides more detailed understanding about carbon and nutrients responses to microbial activities, adding the knowledge of specific mechanisms by which nutrients and labile carbon pool are closely linked to gaseous carbon loss from the waters. This frequent BDOC analysis during incubation is not often seen in other research, therefore the collected data could provide a good basis for additional work to be undertaken to better understand

the dynamics and mutual interaction of carbon and nutrients before BDOC depletion occurs.

With so many future research directions the research field of understanding better DOC composition, as well as concentration in fluvial systems, offers many opportunities.

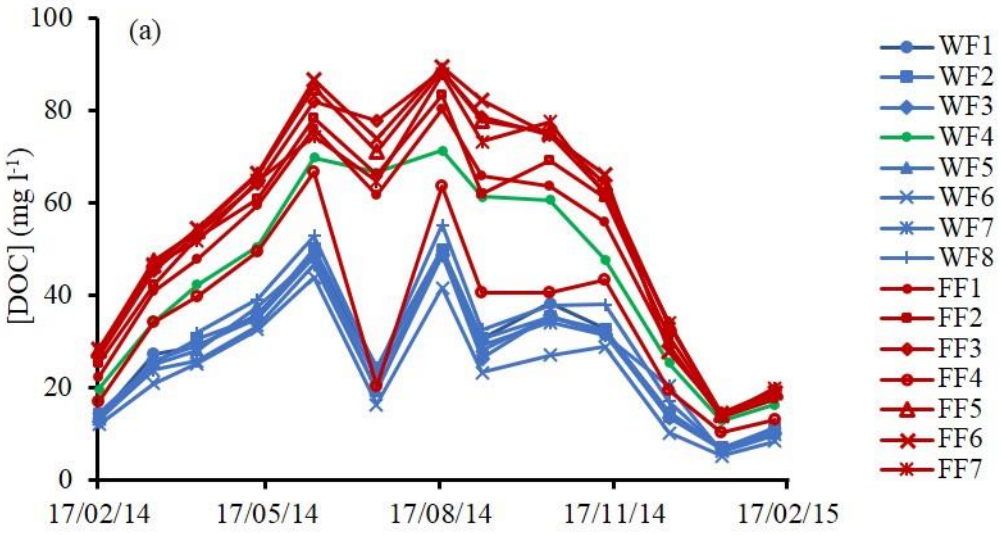
Appendices

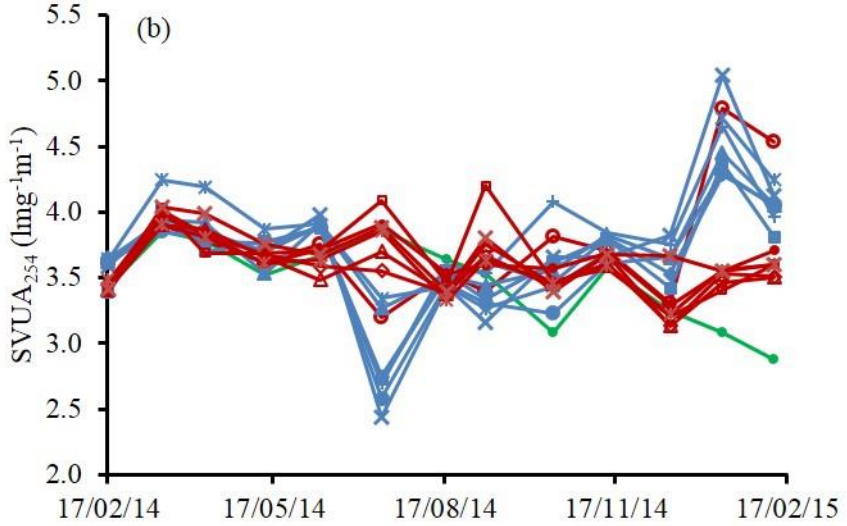
Appendix A – Drumtee data treatment

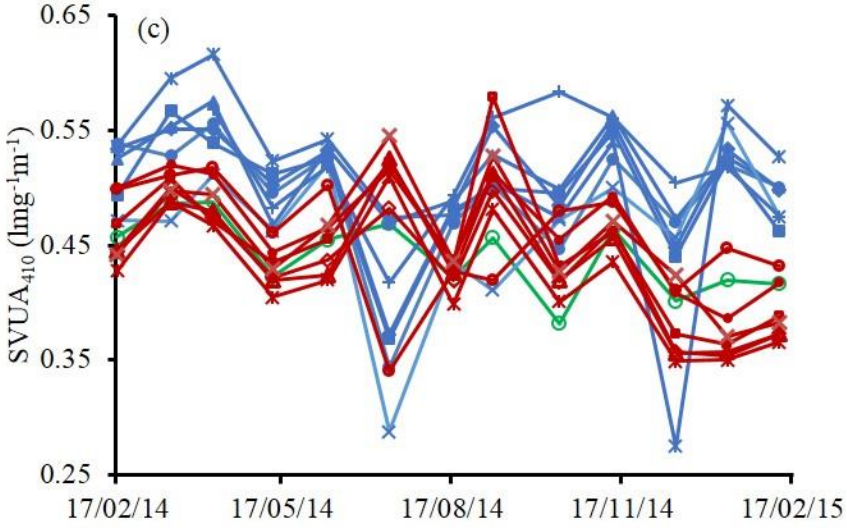
This section contains the time series (Feb. 2014 – Feb. 2015) of DOC concentration and composition for all sites in Drumtee catchment, and the comparison for mean values of the two streams (D-WF and D-FF) when D-WF4 was re-grouped with other D-WF sampling sites (group 1) or with the D-FF sites (group 2). By presenting the primary data, it is demonstrated why D-WF4 was treated as part of D-FF group in the data analysis.

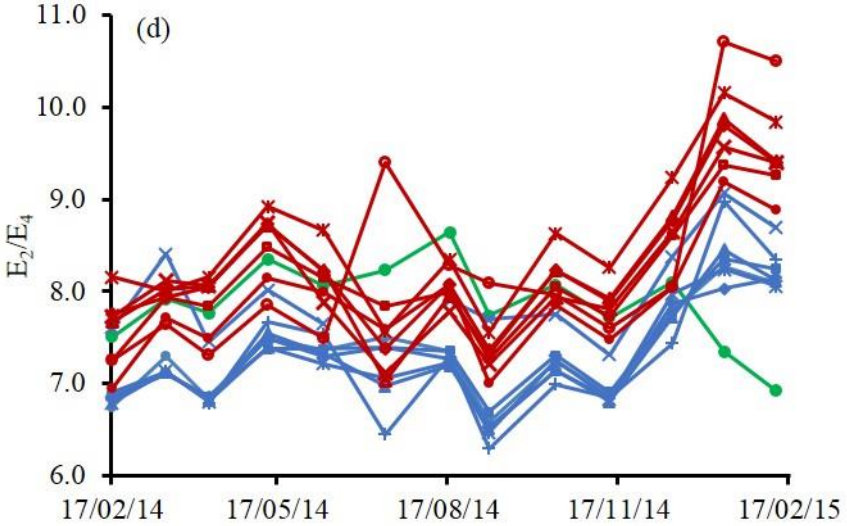
Fig. A-1 shows that for all sampling occasions, both the DOC concentration and composition (represented by SUVAs and E ratios) for D-WF4 are more similar to D-FF site profiles. Fig. A-2 shows that when D-WF4 is considered within the population of other D-WF sites, there is a clear increase of the variation for most parameters (compare the SD errors bars between group 1 and group 2 for D-WF). However, its addition to D-FF hardly changes the population variation. The exact SD changes for D-WF sites with applying group 1 and 2 are presented in Table A-1. From this it can be concluded that the D-WF4 DOM pool is more similar to that of D-FF group, and not the D-WF group.

Further, the catchment land use map (Fig. 2-3) clearly shows that D-WF4 tributary is draining mostly the deforested land. To allow most insight into how DOM compositional changes may be impacted by different land uses, it is considered appropriate to re-grouped D-WF4 with D-FF as these sites are mainly dominated by deforestation.




(b)
SVUA254 (lmg-1m-1)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
17/02/14
17/05/14
17/08/14
17/11/14
17/02/15


(c)
SVUA410 (lmg-1m-1)
0.65
0.55
0.45
0.35
0.25
17/02/14
17/05/14
17/08/14
17/11/14
17/02/15


(d)
E2/E4
11.0
10.0
9.0
8.0
7.0
6.0
17/02/14
17/05/14
17/08/14
17/11/14
17/02/15

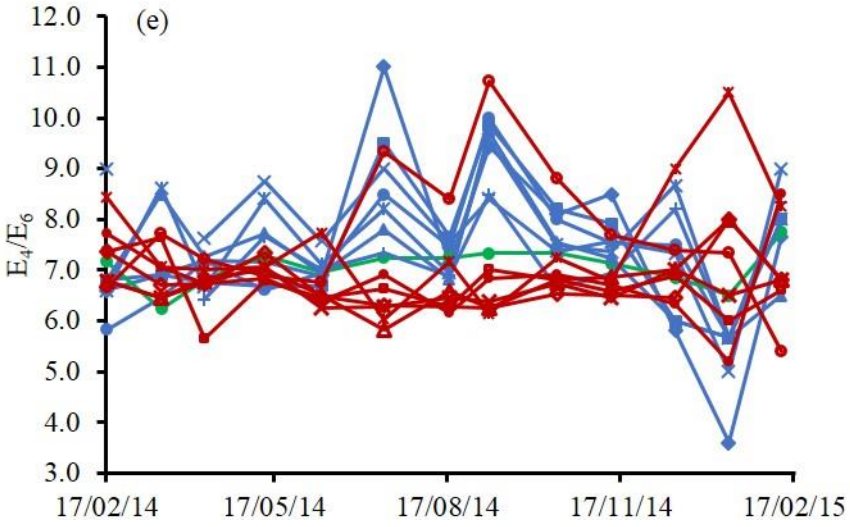
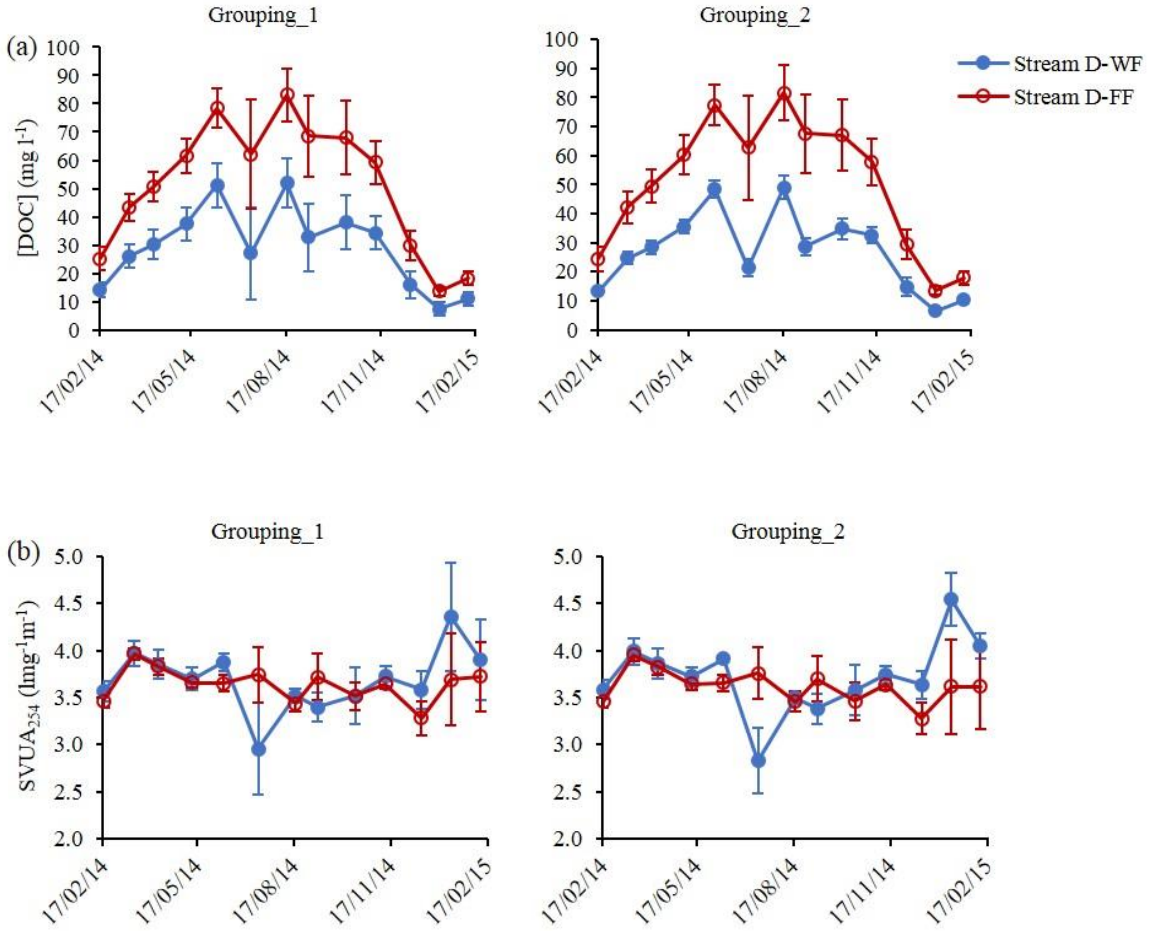


Fig. A-1. DOC concentration, SUVAs and E ratios of all Drumtee samples from both streams during Feb. 2014 – Feb. 2015. The D-FF group is coloured in red and D-WF group in blue. Site D-WF4 is represented by the green empty symbol, and is clearly more similar to D-FF.



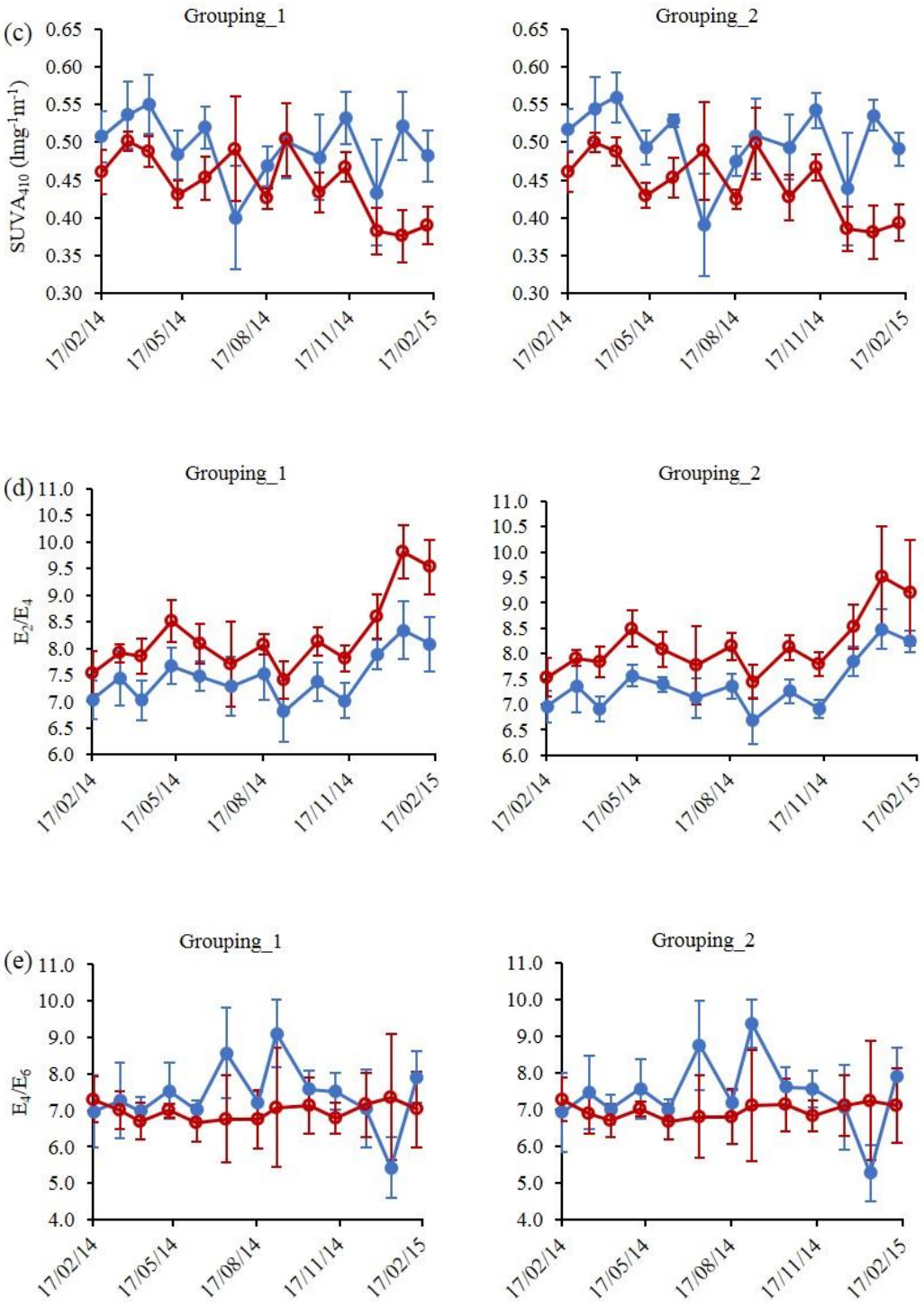


Fig. A-2. The comparisons between different grouping methods. Grouping_1 means no change is applied to the data division. Grouping_2 represents the method used in the data analysis: pooling D-WF4 with D-FF sites. By applying grouping_2, the SDs decreased in [DOC] for the year, and were similar or decreased in different UV-vis parameters for most months. No clear change is observed in D-FF after emerging with D-WF4.

Table A-1 Standard deviations of all parameters measured for D-WF samples. The grouping methods 1 and 2 are the same as described in Fig. A-2. Similar or smaller SDs are coloured in red. By applying method 2, the SDs decreased in [DOC] for the year, and were similar or decreased in UV-vis parameters for most months. SDs for D-FF samples are not presented, as no clear change is observed between different grouping methods.

Parameter	Grouping Method	17/02/14	18/03/14	10/04/14	12/05/14	11/06/14	14/07/14	18/08/14	08/09/14	14/10/14	12/11/14	16/12/14	13/01/15	10/02/15
[DOC]	1	2.5	4.1	5.3	5.7	8.0	16.2	8.7	11.9	9.7	6.0	4.8	2.3	2.3
	2	0.8	2.1	2.4	2.3	2.8	3.0	4.0	3.1	3.7	2.8	3.2	0.7	1.0
$SUVA_{254}$	1	0.1	0.1	0.2	0.1	0.1	0.5	0.1	0.2	0.3	0.1	0.2	0.6	0.4
	2	0.1	0.1	0.2	0.1	0.0	0.3	0.1	0.2	0.3	0.1	0.1	0.3	0.1
$SUVA_{410}$	1	0.03	0.04	0.04	0.03	0.03	0.07	0.03	0.05	0.06	0.03	0.07	0.05	0.03
	2	0.03	0.04	0.03	0.02	0.01	0.07	0.02	0.05	0.04	0.02	0.08	0.02	0.02
E_2/E_4	1	0.36	0.52	0.37	0.34	0.27	0.55	0.50	0.57	0.36	0.33	0.28	0.54	0.51
	2	0.32	0.52	0.24	0.22	0.15	0.39	0.24	0.46	0.24	0.18	0.29	0.39	0.22
E_4/E_6	1	0.98	1.04	0.39	0.76	0.26	1.25	0.32	0.93	0.51	0.49	1.07	0.84	0.70
	2	1.07	1.01	0.41	0.81	0.28	1.22	0.35	0.64	0.54	0.51	1.15	0.78	0.76

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